SIMULTANEOUS ESTIMATION OF OLANZAPINE AND FLUOXETINE HYDROCHLORIDE IN TABLET DOSAGE FORM BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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In partial fulfillment of the requirements for the award of the degree of

MASTER OF PHARMACY

(Pharmaceutical Analysis)

Submitted by

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MAY- 2012.

CERTIFICATE

This is to certify that the dissertation entitled **"SIMULTANEOUS ESTIMATION OF OLANZAPINE AND FLUOXETINE HYDROCHLORIDE IN TABLET DOSAGE FORM BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY''** is a bonafide and genuine research work carried out at Department of Pharmaceutical Analysis, K.K. College of Pharmacy, Chennai – 600122, by **Mr. G. GURUNADHA REDDY** during the academic year 2011-2012 under my direct guidance and supervision. This dissertation submitted in partial fulfillment for the award of the award of **Degree of Master of Pharmacy (Pharmaceutical Analysis)** to The Tamil Nadu Dr. M.G.R Medical University, Chennai – 600032.

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DEDICATED TO MY

BELOVED PARENTS, SISTER

&

ALL MY FRIENDS

LIST OF ABBREVIATIONS USED

°C	-	Degree Celsius
g	-	Grams
ICH	-	International Conference on Harmonization
λ	-	Lambda
LOD	-	Limit of Detection
LOQ	-	Limit of Quantification
µl	-	Microlitre
µ	-	Micron
µg/ ml	-	Microgram per Milliliter
mg/ tab	-	Milligram per tablet
ml	-	Millilitre
nm	-	Nanometer
pH	-	Negative Logarithm of Hydrogen Ion
%	-	Percentage
% RSD	-	Percentage Relative Standard Deviation
RP-HPLC	-	Reverse Phase High Performance Liquid Chromatography
rpm	-	Rotations per Minute
Rt	-	Retention Time
S.D.	-	Standard Deviation

S.E.	-	Standard Error
UV	-	Ultraviolet
USP	-	United States of Pharmacopoeia
v/ v	-	Volume/Volume

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I. INTRODUCTION

The present work deals with the studies carried out on the development, optimization and validation of RP-HPLC method for the simultaneous estimation of Olanzapine and Fluoxetine Hcl in Pharmaceutical dosage form.

Analytical chemistry is a branch of chemistry that deals with the separation, identification and determination of components in a sample. It is the science of making quantitative measurements, which requires background knowledge of chemical and physical concepts[2].

I.I Analytical chemistry

Analytical chemistry may be defined as the "Science and art of determining the composition of materials in terms of the elements or compounds contained" Pharmaceutical analysis plays a major role today, and it can be considered as an interdisciplinary subject. Pharmaceutical analysis derives its principles from various branches of science like Chemistry, Physics, Microbiology, Nuclear Science, Electronics, etc. Analytical method is a specific application of a technique to solve an analytical problem. Analytical instrumentation plays an important role in the production and evaluation of new products and in the protection of consumers and the environment. This instrumentation provides the lower detection limits required to assure safe foods, drugs, water and air.

Pharmaceutical analysis techniques are applied mainly in two areas[1]

Traditionally, analytical chemistry has been split into two main types,

Qualitative Analysis – Information regarding the presence or absence of one or more components of the sample is taken in to account

Quantitative Analysis – Amount of analyte present is determined using appropriate analytical method

Classification of Instrumental Methods of Analysis

The instrumental techniques are classified as

- Spectroscopic Techniques
- Electrochemical Techniques

- Chromatographical Techniques
- Miscellaneous Techniques
- Hyphenated Techniques

A) Spectroscopic Techniques

Spectroscopy is the measurement and interpretation of electromagnetic radiation absorbed, scattered, or emitted by atoms, molecules or other chemical species.

Examples:

- UV Spectrophotometry
- Atomic Spectrometry
- Infrared Spectrometry
- Raman Spectrometry
- X-Ray Spectrometry
- Nuclear Magnetic Resonance Spectrometry

B) **Electrochemical Techniques**

In this, each basic electrical measurement of current like resistance and voltage has been measured alone or in combination for analytical purposes.

Examples:

- Potentiometric Techniques.
- Voltametric Techniques.
- Amperometric Techniques.
- Electrogravimetry.
- Conductance Techniques.

C) Chromatographic Techniques

Chromatography provides versatility in the type of analysis that can be performed. This versatility, due to the wide choice of materials for the stationary and mobile phase, makes it possible to separate molecules that differ only slightly in their physical and chemical properties and can also be quantitatively estimated.

Examples:

- Gas Chromatography
- High performance Liquid Chromatography
- Thin Layer Chromatography

D) Miscellaneous Techniques

Examples:

- Thermal Analysis
- Mass Spectrometry
- Kinetic Techniques

E) Hyphenated Techniques

Separation of mixtures by chromatographic processes is a central part of analytical and preparative chemistry. The direct conjunction of these chromatographic techniques with spectroscopic examination of the separated fractions constitutes several powerful analytical partnerships.

Examples:

- GC-MS (Gas Chromatography – Mass Spectrometry)
- ICP-MS (Inductivity Coupled Plasma Mass Spectrometry)
- GC-IR (Gas Chromatography – Infrared Spectroscopy)
- MS-MS (Mass Spectrometry – Mass Spectrometry)

CHROMATOGRAPHY

Chromatography is a non-destructive procedure for resolving a multi-components mixture of trace, minor, or major constituents into its individual fractions. Different variations may be applied to solids, liquids, and gases. While chromatography may be applied both quantitatively, it is primarily a separating tool.

Russian botanist Michael Tswett invented chromatography as a separation technique. He described in detail the separation of pigments, the colored substances by filtration through column, followed by developments with pure solvents. The first paper of Tswett, was published in 1903, contains a study of more than 100 absorbents used in conjunction with several different solvents.

Other developments in the progress of chromatography were the presentation of gas-liquid chromatography by James and Martin. Despite the exciting possibilities that Tswett's possibilities observation evoked, little work was carried out until 1931 when Kuhn and Lederer repeated Tswett's experiments using alumina and calcium carbonate to separate plant pigments.

The techniques were basically liquid-solid absorption chromatography (LSC); it was not until the late 1930's. In 1941, Martin and Synge introduced liquid-liquid partition chromatography (LLC) and in that publication Martin and Synge also suggested the possibilities of gas chromatography (GC).

In the early 1950's, James and Martin started work on the original suggestion of Martin and Synge developed the first gas chromatograph. Between 1954 and 1962 gas chromatography (GLC) developed at remarkable rate and by 1962, became a mature analytical technique.

The renaissance of LC started in the late 1960's and by the early 1980's LC had also become an established analytical technique used generally for the separation and analysis of a wide range of complex mixtures including many that had previously been analyzed by GC. Some of the more recent developments in LC may well provoke further synergistic developments in GC but further progress in LC appears at this time to be relatively slow just as it has been with over the last two decades.

CLASSIFICATION OF CHROMATOGRPHY

Based on the principle involved

- Absorption Chromatography
- Partition Chromatography
- Ion-exchange Chromatography
- Permeation Chromatography
- Affinity Chromatography
- Electrophoretic Chromatography

Types of Chromatography

Chromatography characterized as a separation method based on the differential migration of solute through a system of two phases, one is mobile phase another one is stationary phase. Chromatography is a technique by which the components in a sample, carried by the liquid or gaseous phase, are resolved by sorption, desorption steps on the stationary phase.

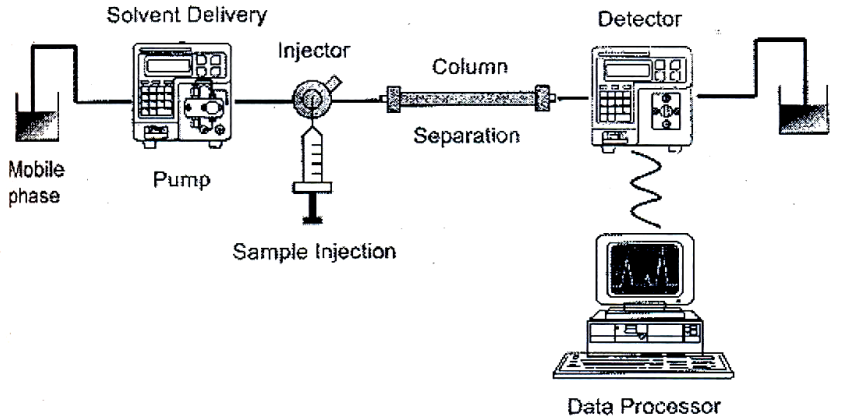
Classification of various types of Chromatographic methods

Technique	Stationary phase	Mobile phase
Column chromatography	Solid	Liquid
Partition chromatography	Liquid	Liquid
Paper chromatography	Liquid	Liquid
Thin layer chromatography	Liquid (or) solid	Liquid
Gas-liquid chromatography	Liquid	Gas

Gas-solid chromatography	Solid	Gas
Ion-exchange chromatography	Solid	Liquid

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

High Performance Liquid Chromatography is the most widely used of all of the analytical separation techniques with the annual sales of HPLC equipment approaching the billion-dollar mark. The reason for the popularity of the method is its sensitivity, its suitability for separating non-volatile or even thermally fragile ones, its ready adaptability to quantitative determinations and above all its wide spread applicability to substances that are of primary interest to industry, to many fields of science, biomedical applications and to the public. Examples of such materials include amino acids, nucleic acids hydrocarbons, carbohydrates, drugs, threnodies, pesticides, antibiotics, and metal organic species. The development of the open-column methods, i.e. paper chromatography (in the 1940s) and thin layer chromatography (in the 1950s), greatly improved the speed and resolution of LC, but there were still serious limitations compared to modern LC methods. In that, analysis times were long, resolution was poor and quantitative analysis, preparative separations and automation were difficult. The rapid growth of HPLC has been facilitated by the development of reliable, moderately priced instrumentation and efficient columns. Now, HPLC analysis is most widely used instrumental method for assays.



Schematic diagram of HPLC

HPLC BASIC INSTRUMENTATION

TYPES OF HPLC

Based on Modes of Chromatography:

- Normal phase chromatography
- Reverse phase chromatography

Based on Principle of Separation:

- Adsorption chromatography
- Ion exchange chromatography
- Size exclusion chromatography
- Affinity chromatography
- Chiral phase chromatography

Base on Elution Technique:

- Isocratic separation
- Gradient separation

Based on the Scale of Operation:

- Analytical HPLC
- Preparative HPLC

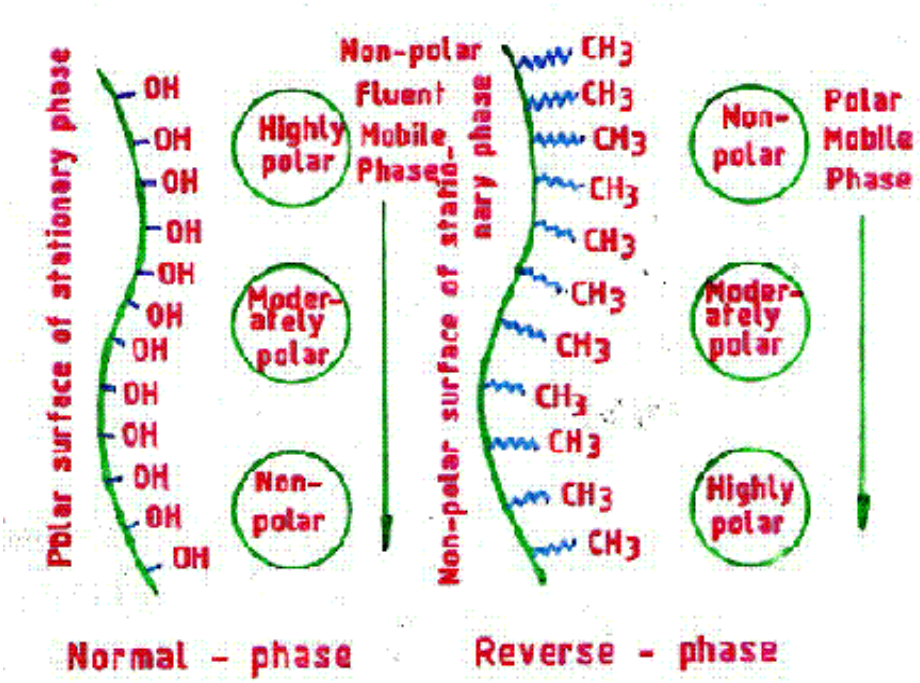
a) Normal phase chromatography

Normal phase chromatography is chromatographic technique that uses organic solvents for mobile phase and a polar stationary phase. Here, the less polar compound elutes faster than the more polar compound.

b) Reverse phase chromatograph since 1960's chromatographers started modifying the polar nature of silanol group by chemically reacting silica with organic silanes. The objective was to make less polar or non-polar so that polar solvents can be used to separate water-soluble polar compounds. Since the ionic nature of the chemically modified silica is now reversed i.e. it is non-polar or the nature of the phase is reversed. The chromatographic separation carried out with such silica is referred to as reversed- phase chromatography.[17]

A large number of chemically bonded stationary phases based on silica are available commercially. There are some of the functional groups bonded in chemically modified silica. Silica based stationary phases are still most popular in reversed phase chromatography however other absorbents based on polymer (styrene-di-vinyl benzene copolymer) are slowly gaining ground.

I. 2 Normal – Reverse Phase Chromatography



As a general rule the retention increases with increasing contact area between sample molecule and stationary phase i.e. with increasing number of water molecules, which are released during the adsorption of a compound. Branched chain compounds are eluted more rapidly than their corresponding normal isomers.

In reversed phase systems the strong attractive forces between water molecules arising from the 3 - dimensional inter molecular hydrogen bonded network, from a structure of water that must be distorted or disrupted when a solute is dissolved. Only higher polar or ionic solutes can interact with the water structure. Non- polar solutes are squeezed out of the mobile phase and are relatively insoluble in it but with the hydrocarbon moieties of the stationary phase, they get adsorbed.

Reverse phase chromatography - a bonded phase chromatography technique, uses water as base solvent. Separation is based on solvent strength and selectivity. Separation is also affected by column temperature and pH. In general, the more polar compounds elute faster than the less polar compounds. UV detection is the most common detection technique used.[3]

Certain limitations of RP-HPLC are:

- Compounds much more polar than the compound of interest may be masked (eluted together) in the solvent front / void volume.
- Compounds very less polar than the analyte may elute either late during the chromatographic run or are retained in the column.
- The compounds with lower UV extinction coefficients or different wavelengths maxima may not be detectable at the low level relative to the visibility of the analyte since only one or two wavelengths are monitored.

c) Size exclusion chromatography

It is also known as gel permeation or filtration chromatography. Here, separation is based on the molecular size or hydrodynamic volume of the components. The stationary phase is a wide pore gel that can separate molecules on the basis of their size and shape, the largest molecules traveling most rapidly through the system. So, molecules that are too large for the pores of the porous packing material on the column elute first, small molecules that enter the pores elute last, and the elution rates of the rest depend on their relative size

d) Ion Exchange Chromatography

The stationary phase is an ion exchange resin, and separations are governed by the strength of the interactions between solute ions and the exchange sites on the resin. An ion exchange resin consists of an insoluble, rigid three-dimensional matrix, for example polystyrene cross-linked with a small amount of divinylbenzene to produce mechanical stability. The surface of this matrix contains ionizable sites that can carry a positive or a negative charge. Each of these sites also require an oppositely charged ion for overall neutrality. If the ionizable sites are positively charged, the counter ion is an anion and the resin will exchange anions from solution.

e) Ion pair /Affinity chromatography

Separation is based on chemical interaction, specific to the target species. The more popular reverse phase mode uses a buffer and an added counter ion of opposite charge to the sample with separation being influenced by pH, ion strength, temperature, concentration and organic modifier. Affinity chromatography, commonly for macromolecules employs a ligand

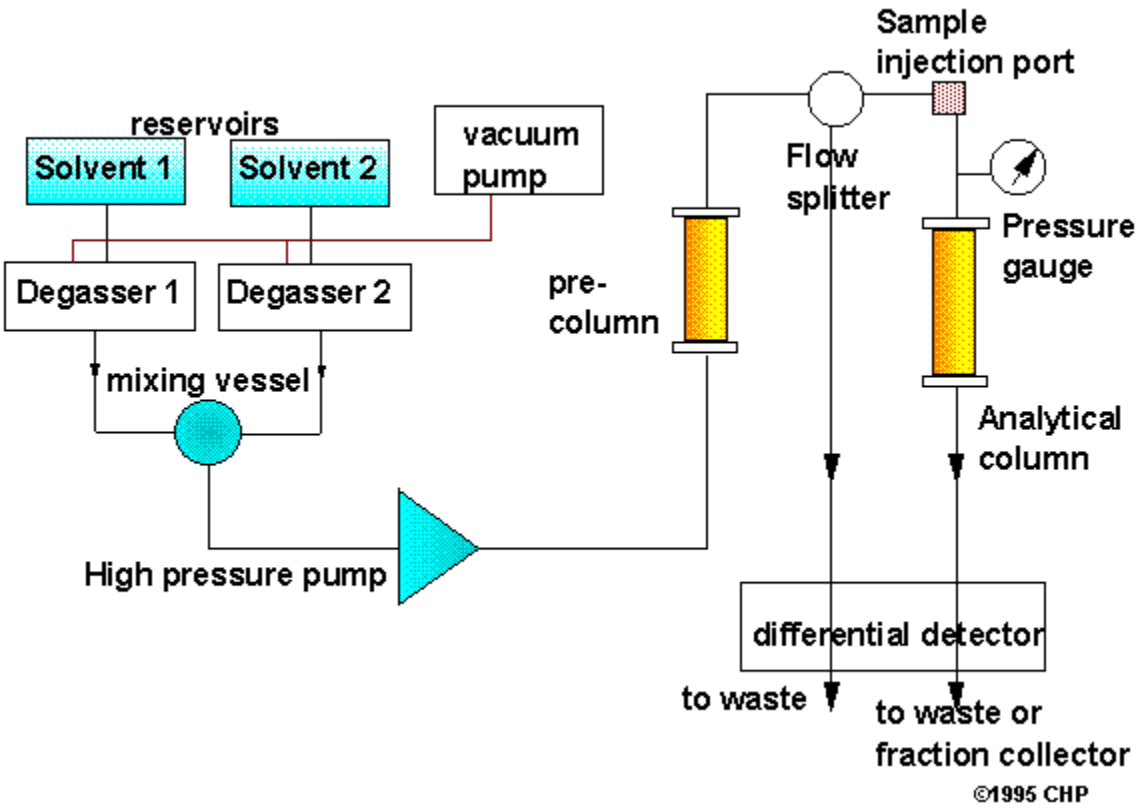
(biologically active molecule bonded covalently to the solid matrix), which interacts with its homologous antigens as a reversible complex that can be eluted by changing buffer conditions.

f) Chiral Chromatography

Chirality plays an important role in pharmaceutical industry. It is mainly because of the enantiomers exhibit different pharmacological and toxicological properties in living systems. A tragic example was thalidomide, a sedative and sleep inducing drug, which caused serious malformations in new borns of women who consumed it during an early phase of pregnancy. Later, it was realized that S-(-) enantiomer of thalidomide possess teratogenic action and has no importance for the desired sedative or sleep inducing property. Very often the enantiomers of racemic drugs possess different pharmacological effects. For example, S-(-)-propranolol is considerably more active than its R-(+) enantiomer. The anesthetic ketamine is generally administered as a racemate.

Advantages and disadvantages of the mobile phase additives

Advantages	**Disadvantages**
1. Less expensive conventional LC columns can be used 2. A wide variety of possible additive are available 3. Different selectivities from the chira phases can be obtained.	1. Many chiral additives are costly and sometimes have to be synthesized. 2. The mode of operation is complex 3. Inconvenient for preparative applications because the chiral additive must be removed from the Enantiomeric solutes.



Schematic diagram of High Performance Liquid Chromatography

INSTRUMENTATION:

The essential parts of the High Performance Liquid Chromatography are:

- Solvent reservoir
- Mobile phase
- Pump system
- Sample Injection System
- Column
- Detector

Solvent reservoir

A modern HPLC apparatus is equipped with one or more glass or stainless steel reservoirs. The reservoir is often equipped with an online degasser which removes the dissolved gasses usually oxygen and nitrogen, which interfere by forming bubbles. Degasser may consist of vacuum pumping system, distillation system, system devices for heating, and solvent stirrer.

Mobile phase

One of the greatest advantages of HPLC is versatility afforded by liquid mobile phase. Not only can different parameter be varied when the mobile phase is liquid, but also the solute can interact with the mobile phase as well as with the stationary phase. Sufficient solubility of solute molecules in the mobile phase must be ensured in order to prevent precipitation.

Pumping system

The function of the pump in HPLC is to pass mobile phase through the column at a controlled flow rate. Features of an ideal pumping system include:

Generating pressure from 6000 psi to 10000 psi.Pulse free output.

Flow rates ranging from 0.1 to 10 ml/min. Flow control and reproducibility of 0.5% relative or better. Corrosion resistant components.

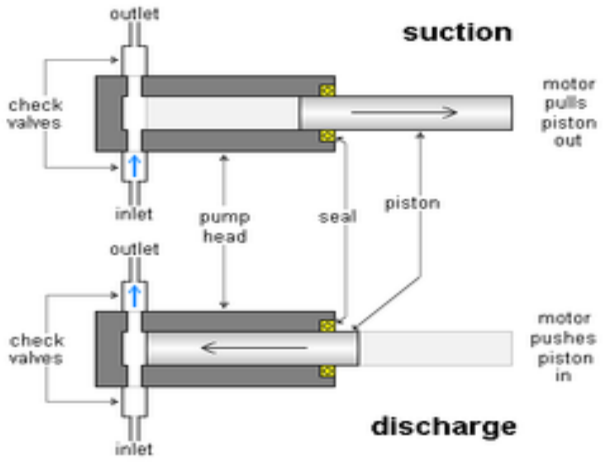
HPLC pump can be classified in to the following groups according to the manner in which they operate:

- Constant flow rate pump (or) constant displacement pump
    - i) Reciprocating piston pump
    - ii) Syringe drive pump
- Constant pressure pump
    - i) Simple gas displacement pump
    - ii) Pneumatic amplifier pump

1. Reciprocating pump

Reciprocating pumps usually consist of a small chamber in which the solvent is pumped by the back and forth motion of a motor driven piston. Two check valves control the flow of solvent. Reciprocating pumps have a disadvantage of producing pulsed flow, which must be damped as its presence is manifested as base line noise on the chromatogram. Advantages of this pump include their small internal volume, high output pressure, ready adaptability to gradient elution, and independent of column backpressure and viscosity of solvent

Piston pumps



2. Displacement pump

Displacement pumps usually consist of large syringe like chambers equipped with a plunger that is activated by a screw driver mechanism powered by stepping motor. Displacement pumps also produce a flow that tends to be independent of viscosity and backpressure. In addition, the output is pulse free. Disadvantages include limited solvent capacity (250 ml) and considerable inconvenience when solvents must be changed.

3. Pneumatic pumps

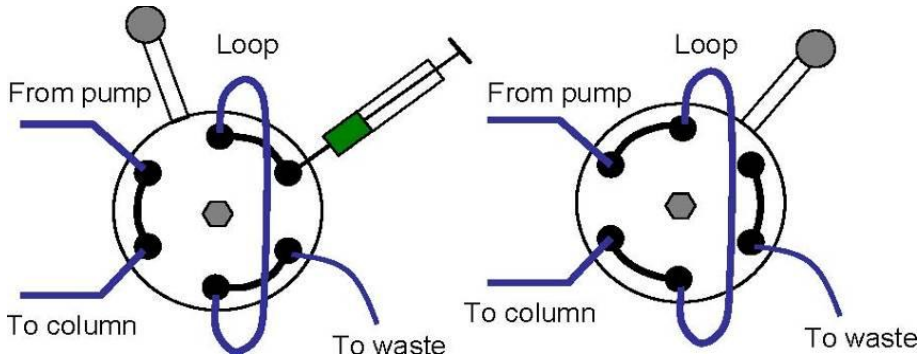
In pneumatic pumps, the mobile phase is contained in a collapsible container housed in a vessel that can be pressurized by a compressor gas. Pumps of this kind are inexpensive and pulse free. They suffer from limited capacity, pressure output, dependence of flow rate on

solvent viscosity and column backpressure. In addition, they are not amenable to gradient elution and are limited to pressures less than about 2000 psi.

Sample injection system

The limiting factor in the precision of LC measurements lie in reproducibility with which samples are introduced into the column packing. The earliest and simple means of sample introduction was syringe injection through a self-sealing electrometric septum. In stop flow injections, the flow of solvent is stopped momentarily, and fitting at column head is removed and the sample is injected directly into the head of column packing. After replacing the fitting the system is again pressurized.

Sample injection system



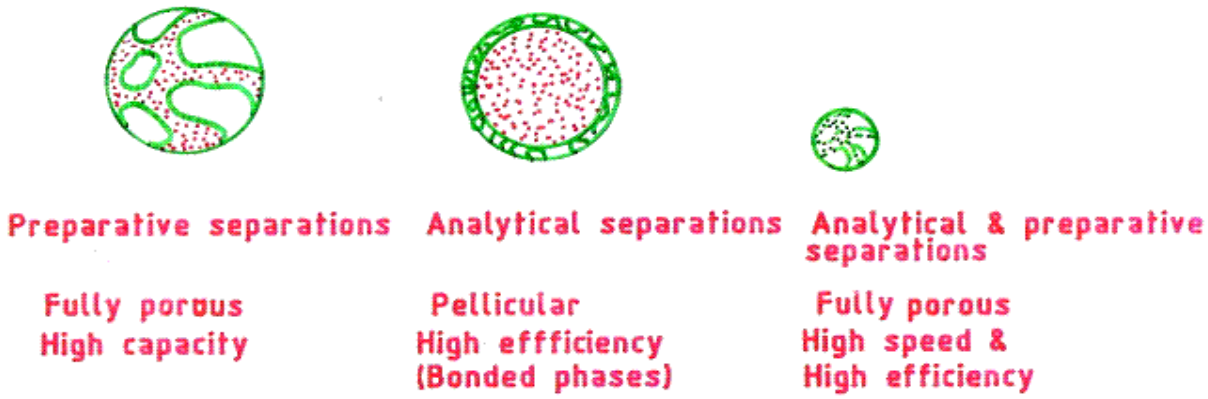
Columns

The columns most commonly used are made with 316-grade stainless steel (a Cr-Ni-Mo steel, relatively inert to chemical corrosion). The inside of the stainless steel tube should be as smooth as possible, so the tubes are precision drilled or electro-polished after manufacture. Common dimensions are 6.35 mm external diameter, 4.6 mm internal diameter and up to 25 cm long. The columns can be packed with 10, 5, 4 or 3 µm diameter particles.

At the top of the column, there is a distributor for directing the injected sample to the center of the column and then a stainless steel gauze or frit on top of the packing. At the lower

end there is another frit to retain the packing, and then, for the 4.6 mm type, a reducing union and a short length of 0.25 mm (0.01 in.) i.e. tubing to connect the column to the detector.

Materials other than stainless steel that are used for columns include glass, glass lined steel tube and polyethene or other inert plastics.



Properties of particles in various types of columns

1 Analytical columns

The majority of LC columns range in length from 5 to 30 cm. The inner diameter of liquid columns is often 4 to 5 mm. Most common particle size of packing is 5 to 10 µm. Columns of this type contain 40000 - 60000 plates per meter

2 Preparative columns

Preparative columns are typically 2-5 cm in diameter and 25 cm long with packing of 15-100-µm diameter. Columns for large-scale work can be 20-30 cm in diameter and 60 cm long, using flow rates up to 1000 cc min-1. The commercial systems can be used isocratically or with gradients, and allow small-scale development and preparative separation to be done using the same system.

3 Guard columns

Usually, a short guard column is introduced before the analytical column to increase its life. It removes particulate matter, contaminants from the solvents and also sample components that bind irreversibly to stationary phase. The composition of the guard column packing should be closely similar to that of analytical columns

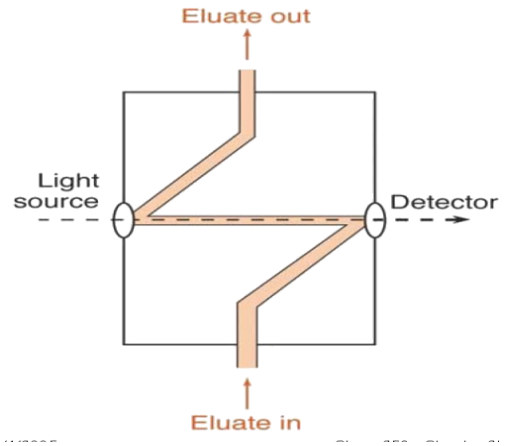
Partisil® High performance liquid chromatography (HPLC) columns

Detectors

The function of the detector in HPLC is to monitor the mobile phase emerging from the column. The output of the detector is an electrical signal that is proportional to some property of the mobile phase and/or the solutes.

LC detectors are basically of two types.

LC Detector



Bulk property detectors respond to mobile phase bulk property such as refractive index, dielectric constant or density.

Solute property detectors respond to some property of solutes, such as UV absorbing, fluorescence, diffusion current, which are not possessed by the mobile phase.

Most common HPLC detectors

- UV-Visible absorbance detector (UV-VIS)
- Photo-diode array detector (PDA)
- Fluorescence detector
- Electrochemical (ECD)
- Refractive Index (RI)
- Mass detectors (MS)
- Conductometric detector
- Chiral detector (Polarimetric & circular dichrosim)
- Evaporative Light scattering detector (ELSD)
- Radiochemical detection

UV Detector

1. UV absorbing detector

The general applicability and ease of operation of UV-Visible absorbance detectors have made it most popular in LC. It measures the absorbance of the monochromatic light by the solute according to the Beer-Lambert's law.

Where, 'I' is the intensity of the transmitted light, 'Io' is intensity in the absence of the analyte. 'a' is molar absorpitivity of analyte at the wavelength used, 'b' is the length of the optical cell used and 'c' is the concentration of the analyte

$$I = I0\ 10^{-abc}$$

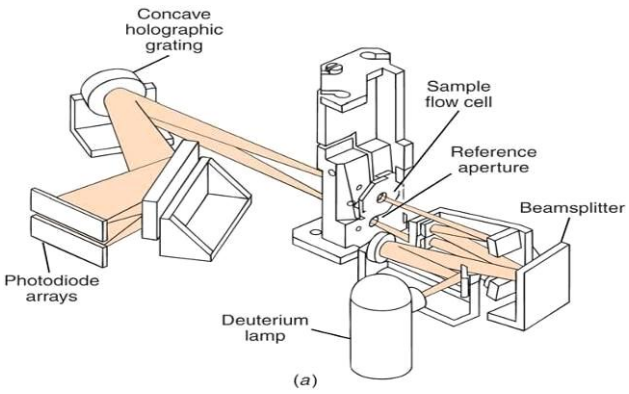
2. Photodiode array detectors

In Photo Diode Array detector, the single detecting of UV detector has been replaced by an array of solid state detecting elements (photo-diodes). These detectors typically have large numbers of diodes in array (256, 512, and 1024). In PDA, polychromatic radiation, after passing through the sample, is dispersed by a fixed grating and then falls on to an array of photodiodes. Each diode measures a narrow band of wavelengths in the spectrum, thus the PDA has parallel data acquisition, all points in the spectrum being measured simultaneously. The spectrum of each peak in the chromatogram can be stored and subsequently compared with standard spectra, which facilitates the identification of peaks.

This system is superior to other detection systems as:

There are no moving parts to wear out, wavelength-resetting errors are reduced and the instrument is likely to require less maintenance than does a conventional spectrophotometer.

The ability to make multiwavelength measurements and the speed of data acquisition mean that various signal-averaging techniques can be used to reduce noise and improve sensitivity.



Photodiode array detector

3 Peak purity by PDA detector:

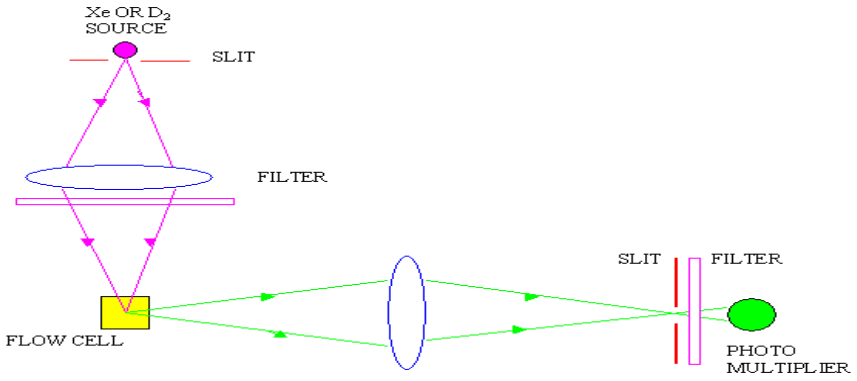
When two compounds elute very close to each other, they appear as one peak. This can be recognized by a difference of the spectra recorded on the two flanks of the peak, provided the two compounds have different absorption spectra. When PDA is combined with a separating column, three dimensions become available; wavelength, intensity and chromatographic retention. The spectra obtained must be sufficiently different as specified by their correlation coefficients. Even with a value under 0.9, superimposed peaks can be calculated and evaluated in a three dimensional space without substantial loss in terms of accuracy and precision to know the purity of a particular peak.

4. Fluorescence detectors

Many compounds are capable of absorbing UV radiation and subsequently emitting radiation of a longer wavelength, either instantly (fluorescence) or after a time delay (phosphorescence). Such compounds are suitable for fluorescent detection.

Radiation from a xenon or deuterium source is focused on the flow cell. The simplest detectors employ a mercury excitation source and one or more filters to isolate the band of

emitted radiation. An interchangeable filter allows different excitation wavelengths to be used. The fluorescent radiation is emitted by the sample in all directions, but is usually measured at 90º to the incident beam. FD for HPLC is similar in design to the fluorometers or spectrofluorometer.



Block diagram of Fluorescence detector

5. Refractive index detectors

RI detectors are bulk property detectors in which the solvent passes through one half of the cell on its way to the column. A glass plate mounted at an angle such that bending of the incident beam occurs if the two solutions differ in refractive index separates the two

Compartments. The resulting displacement of the beam with respect to the photosensitive surface of the detectors causes variation in the output signal.

6. Evaporative light scattering detectors (ELSD)

Evaporative Light Scattering Detection is the preferred concentration detection method for Liquid Chromatography. Their principal requirement is that the analyte should be less volatile than the mobile phase. An ELSD cannot detect highly volatile analytes. In this detector, the column effluent is passed into a nebuliser where, it is converted into fine mist by a flow of nitrogen. The fine droplets are then carried through a controlled temperature drift tube where,

evaporation of mobile phase occurs leading to the formation of fine particles of the analyte. The cloud of analyte particles then passes through a laser beam. The scattered radiation is detected at right angle to the flow by a silicon photo diode.

7. Electrochemical detectors

Electrochemical detection (ECD) is based on the transfer of electrons between either oxidisable / reducible molecule in the solution and a solid conductor. ECD measure either the conductance of the eluent or the current associated with the oxidation or reduction of solutes. To be capable of detection using the first method the solutes must be ionic, and using the second method the solutes must be relatively easy to oxidize or reduce.

8. Conductivity Detectors

The conductivity detectors are used for the detection of inorganic or organic ions, usually after separation by ion exchange chromatography. These detectors oxidize or reduce only a small quantity of the solute, so the currents observed are very small (nanoamps).

ANALYTICAL METHOD DEVELOPMENT

Methods are developed for new products when no official methods are available. Alternate methods for existing (non-Pharmacopoeia) products are developed to reduce the cost and time for better precision and ruggedness. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit / demerits are made available.

Steps of method development

Documentation starts at the very beginning of the development process, a system for full documentation of the development studies must be established. All data relating to these studies must be recorded in laboratory notebook or an electronic database.

1. Analyte standard characterization

All known information about the analyte and its structure is collected i.e., physical and chemical properties.

The standard analyte (≈100% purity) is obtained. Necessary arrangement is made for the proper storage (refrigerator, dessicators, and freezer).

When multiple components are to be analyzed in the sample matrix, the number of components is noted, data is assembled and the availability of standards for each one is determined.

Only those methods (spectroscopic, MS, GC, HPLC etc.,) that are compatible with sample stability are considered.

2. Method requirements

The goals or requirements of the analytical method that need to be developed are considered and the analytical figures of merit are defined. The required detection limits, selectivity, linearity, range, accuracy and precision are defined.

3. Literature search and prior methodology

The literature for all types of information related to the analyte is surveyed. For synthesis, physical and chemical properties, solubility and relevant analytical methods. Books, periodicals, chemical manufacturers and regulatory agency compendia such as USP / NF, AOAC and ASTM publications are reviewed. Chemical Abstracts Service (CAS) automated computerized literature searches are convenient.

4. Choosing a method

Using the information in the literatures and prints, methodology is adapted. The methods are modified wherever necessary. Sometimes it is necessary to acquire additional instrumentation to reproduce, modify, improve or validate existing methods for in-house analytes and samples.

If there are no prior methods for the analyte in the literature, from analogy, the compounds that are similar in structure and chemical properties are investigated and are worked out. There is usually one compound for which analytical method already exist that is similar to the analyte of interest.

5. Instrumental setup and initial studies

The required instrumentation is setup. Installation, operational and performance qualification of instrumentation using laboratory standard operating procedures (SOP's) are verified.

Always new consumables (e.g. solvents, filters and gases) are used, for example, method development is never started, on a HPLC column that has been used earlier.

The analyte standard in a suitable injection / introduction solution and in known concentrations and solvents are prepared. It is important to start with an authentic, known standard rather than with a complex sample matrix. If the sample is extremely close to the standard (e.g., bulk drug), then it is possible to start work with the actual sample.

6. Optimization

During optimization one parameter is changed at a time, and set of conditions are isolated, rather than using a trial and error approach. Work has been done from an organized methodical plan, and every step is documented (in a lab notebook) in case of dead ends.

7. Documentation of analytical figures of merit

The originally determined analytical figures of merit limit of quantitation (LOQ), Limit of detection (LOD), linearity, time per analysis, cost, sample preparation etc., are documented.

8. Evaluation of method development with actual samples

The sample solution should lead to unequivocal, absolute identification of the analyte peak of interest apart from all other matrix components.

9. Determination of percent recovery of actual sample and demonstration of quantitative sample analysis

Percent recovery of spiked, authentic standard analyte into a sample matrix that is shown to contain no analyte is determined. Reproducibility of recovery (average +/- standard deviation) from sample to sample and whether recovery has been optimized has been shown. It is not necessary to obtain 100% recovery as long as the results are reproducible and known with a high degree of certainty.

The validity of analytical method can be verified only by laboratory studies. Therefore documentation of the successful completion of such studies is a basic requirement for determining whether a method is suitable for its intended applications.

Method Validation

Validation

Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications

Validation is defined as follows by different agencies

Food and Drug administration (FDA)

Establishing documentation evidence, which provides a high degree of assurance that specific process, will consistently produce a product meeting its predetermined specification and quality attributes.

World Health Organization (WHO)

Action of providing that any procedure, process, equipment, material, activity, or system actually leads to the expected results

European Committee

Action of providing in accordance with the principles of good manufacturing practice that any procedure, process, equipment, material, activity or system actually leads to the expected results. In brief validation is a key process for effective Quality Assurance.

Reasons for Validation

There are two important reasons for validating assays in the pharmaceutical industry. The first, and by for the most important, is that assay validation is an integral part of the quality-control system. The second is that current good manufacturing practice regulation requires assay validation.

Steps followed for validation procedures

1. Proposed protocols or parameters for validations are established.

2. Experimental studies are conducted.

3. Analytical results are evaluated.

4. Statistical evaluation is carried out.

5. Report is prepared documenting all the results.

Objective and Parameters of Analytical Method Validation

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. According to ICH guidelines, typical analytical performance characteristics that should be considered in the validation of the types of methods are

(a) Recovery

(b) Response function

(c) Sensitivity

(d) Precision

(e) Accuracy

(f) Limit of detection

(g) Limit of quantitation

(h) Ruggedness

(i) Robustness

(j) Stability

(k) System Suitability

(l) Repeatability

(m) Specificity

(a) Recovery

The absolute recovery of analytical method is measured as the response of a processed spiked matrix standard expressed as a percentage of the response of pure standard, which has not been subjected to sample pre-treatment and indicates whether the method provides a response for the entire amount of analyte that is present in the sample. It is best established by comparing the responses of extracted samples at low, medium and high concentrations in replicates of at least 6 with those non-extracted standards, which represent If an internal standard is used, its recovery should be determined independently at the concentration levels used in the method.

$$\text{Absolute Recovery} = \frac{\text{Response an analyte spike into matrix (processed)}}{\text{Response of analyte of pure standard (unprocessed)}} \times 100$$

(b) Response of function

In chromatographic methods of analysis, peak area or peak height may be used as response function to define the linear relationship with concentration known as the calibration model. It is essential to verify the calibration model selected to ensure that it adequately describes the relationship between response function (Y) and concentration (X).

(c) Sensitivity

The method is said to be sensitive if small changes in concentration cause large changes in response function. The sensitivity of an analytical method is determined from the slope of the calibration line. The limits of quantification (LOQ) or working dynamic range of bio analytical method are defined as the highest and lowest concentrations, which can determined with acceptable accuracy.

It is suggested that, this be set at + 15% for both the upper and lower limit of quantitation respectively. Any sample concentration that falls outside the calibration range cannot be interpolated from the calibration line and extrapolation of the calibration curve is discouraged. If the concentration is over range, the sample should be diluted in drug-free matrix and re-assayed.

(d) Precision

The precision of an analytical method is a measure of the random error and is defined as the agreement between replicate measurements of the same sample. It is expressed as the percentage coefficient of variation (%CV) or relative standard deviation (RSD) of the replicate measurements.

$$\%CV = \text{Standard deviation/Mean} \times 100$$

Precision can be considered as having a within assay batch component or repeatability which defines the ability to repeat the same methodology with the same analyst, using the sesame equipment and same reagents in a short interval of time, e.g. within a day. This is also known as intra-assay precision.

The ability to repeat the same methodology under different conditions, e.g. change of analyst, reagent or equipment, or on subsequent occasions, e.g. across several weeks or months, is covered by the between batch precision or reproducibility, also known as inter-assay precision. The reproducibility of a method is of most interest to the analyst since this will give better representation of the precision during routine use as it includes the variability from many sources.

(e) Accuracy

Accuracy normally refers to the difference between the mean x****, of the set of results and the true to correct value of the quantity measured. According to IUPAC accuracy is to the difference between results (or mean) and the true value. For analytical methods, there are two possible ways of determining the accuracy, absolute method and comparative method.

Accuracy is best report as percentage bias, which is calculated from the expression

$$\%Bias = \frac{(\text{Measured value} - \text{True value})}{\text{True value}} \times 100$$

Since for real samples the true value is not known, an approximation is obtained based on spiking drug – free matrix to a nominal concentration. The accuracy of analytical method is then determined at each concentration by assessing the agreement between the measured and nominal concentrations of the analytes in the spiked drug – free matrix sampler.

(f). Limit of detection (LOD)

The limit of detection (LOD) of an analytical method may be defined as the concentration, which gives rise to an instrument signal that is significantly different from the blank. For spectroscopic techniques or other methods that rely upon a calibration curve for quantitative measurements, the IUPAC approach employs the standard deviation of the intercept (Sd), which may be related to LOD and the slope of the calibration curve, b

$$LOD = 10 \text{ x Standard deviation/slope}$$

(g). Limit of Quantitation (LOQ)

The LOQ is the Concentration that can be quantitate reliably with a specified level of accuracy and precision. The LOQ represent the Concentration of analyte that would yield a signal-to-noise ratio of 10.

$$LOQ = 10 \times \text{Standard deviation /slope (or) Sa /b}$$

Where Sd- the estimate is the standard deviation of the peak are ratio of analyte to IS (5 injections) of the drugs. b -is slope of the corresponding calibration curve.

Based on Standard Deviation of the blank

Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.

Based on the calibration curve

A specific calibration curve should be studied using samples, containing an analyte in the range of QL. The residuals SD of regression line or the S.D of intercepts of regression lines may be used as the S.D. The quantitative limit is a parameter of quantitative assay for low levels of compounds in sample matrices, and is use particularly for the determination of impurities or degradation products.

h). Ruggedness

Degree of reproducibility of test results obtained by the analysis of the same samples under a variety of condition such as different laboratories, different analysts, different instruments etc, normally expressed as the lack of influence on test results of operational and environmental variable of the analytical method.

Ruggedness is a measurement of reproducibility of test results under the variation in condition normally expected from laboratory to laboratory and from analyst to analyst. Degree of representative of test results is then determined as a function of the assay variable.

By analysis of aliquots from homogenous lots in different laboratories, by different analyst, using operational and environmental conditions that may differs but are still within the specified parameter of the assay variable.

i). Robustness

Robustness of an analytical method is measure of its capacity to remain unaffected small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Testing varying some or all condition:

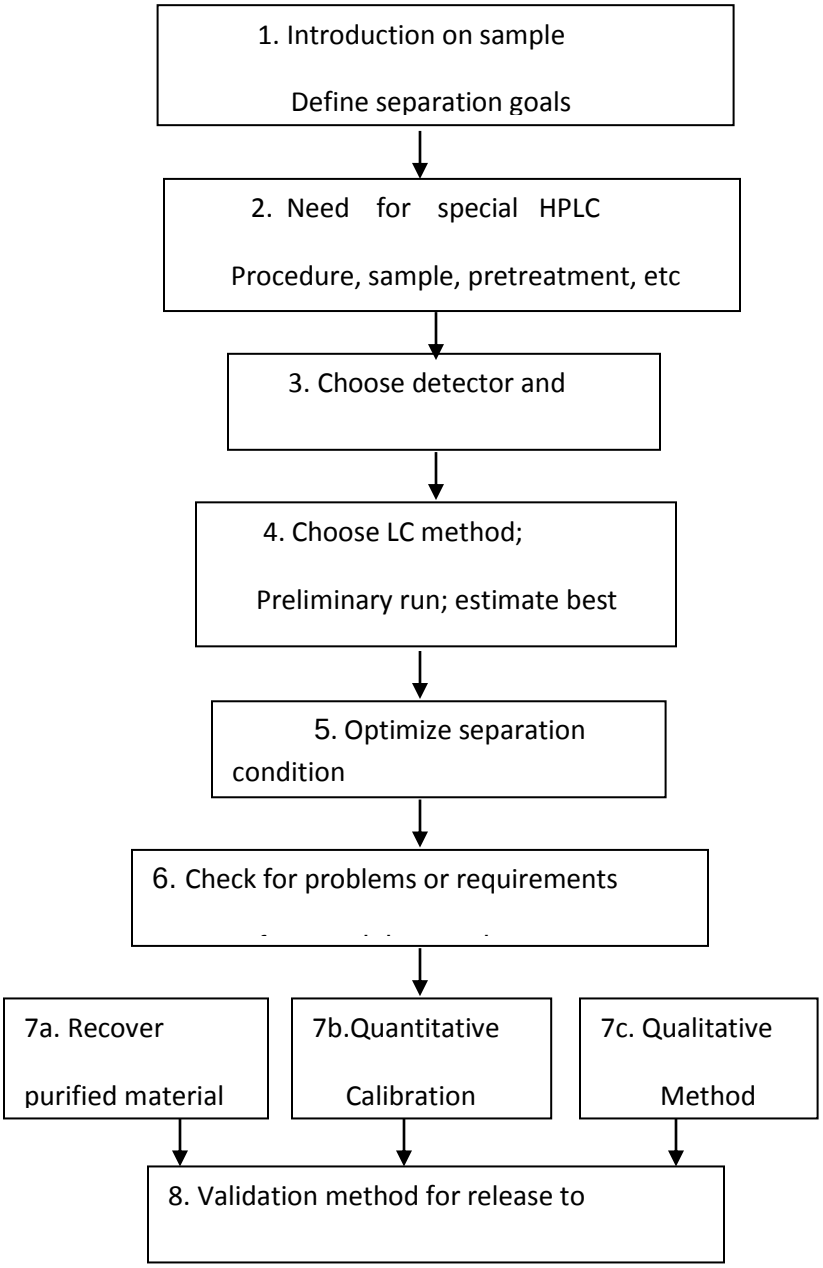
-Column temperature

-PH of buffer in mobile phase

-Reagents and flow rate

HPLC method validation: Everyday many chromatographers face the need to develop a HPLC separation whereas individual approaches may exhibit considerable diversity; method development often follows the series of steps summarized in the following fig

Steps involved in HPLC method validation



II. Review of Literature

1. **J.J. Berzas Nevado et al.,(2000)[14]** Developed Method development and validation for the simultaneous determination of fluoxetine and fluvoxamine in pharmaceutical preparations by capillary electrophoresis Optimal conditions for the quantitative separation were investigated. A background electrolyte solution consisting of 40mMborate buffer adjusted to pH 9.3, hydrodynamic injection and 8 kV of separation voltage were used, obtaining in these conditions analysis times lower than 2.5 min. Main aspects of the validation method are examined and discussed. Detection limits of 1.0 mg/l for fluoxetine and fluvoxamine were obtained.

2. **Ibrahim a. Darwish et al.,(2005)[15]** Developed Three simple and sensitive spectrophotometric Methods were developed and validated for determination of the hydrochloride salts of fluoxetine, sertraline, and paroxetine in their pharmaceutical dosage forms. These methods were based on the reaction of the *N*-alkyl vinyl amine formed from the interaction of the free secondary amino group in the investigated drugs and acetaldehyde with each of 3 haloquinones, i.e.,chloranil, bromanil, and 2,3-dichloronaphthoquinone,to give colored vinyl amino-substituted quinones.The colored products obtained with chloranil ,bromanil, and 2,3-dichloronaphthoquinone exhibit absorption maxima at 665, 655, and 580 nm, respectively.

3. **Kanakapura Basavaiah et al.,(2008)[6],** Carried out A new high performance liquid chromatographic (HPLC)method in reverse phase was developed and validated for the determination of olanzapine (OLZ) in pharmaceutical formulations. Optimum separation was achieved in less than 10 min using a reversed phase Intersil ODS column (150 mm × 4.6 mm, i.d.,particle size 5 mm), and elution was accomplished using a mobile phase (0.5 mL/min). Detection was carried out using a UV detector set at 271 nm. A rectilinear relationship between mean peak area and concentration of OLZ was observed in the range 10-200mg/mL, with a detection limit of 3.0 mg/mL and a quantization limit of 8.0 mg/mL. Intra-day and Inter-day Precision, and accuracy of the methods have been established according to the current ICH guidelines.

4. **CR Shah et al.,(2008)**[9] Carried out A rapid, selective and stability-indicating high performance thin layer chromatographic method was developed and validated for the simultaneous estimation of olanzapine and fluoxetine in combined tablet dosage form. Olanzapine and fluoxetine were chromatographed on silica gel 60 F_{254} TLC plate using methanol: toluene (4:2 v/v) as the mobile
Phase and spectro densitometric scanning-integration was performed at a wavelength of 233 nm using a Camag TLC Scanner III. This system was found to give compact spots for both olanzapine (R_f value of 0.63±0.01) and fluoxetine (R_f value of 0.31±0.01). The polynomial regression data for the calibration plots showed good linear relationship with r^2 =0.9995 in the concentration range of 100-800 ng/spot for olanzapine and 1000-8000 ng/spot for fluoxetine with r^2 =0.9991. The method was validated in terms of linearity, accuracy, precision, recovery and specificity.

5. Sejal Patel and N. J. Patel et al.,(2009)[22] Developed Simultaneous RP-HPLC and HPTLC Estimation of Fluoxetine Hydrochloride and Olanzapine in Tablet Dosage Forms A binary mixture of fluoxetine HCl and olanzapine was determined by two different methods. The first method involved determination of fluoxetine HCl and olanzapine using reversed-phase liquid chromatography using acetonitrile: methanol: 0.032 M ammonium acetate buffer (45:05:50, v/v/v) as the mobile phase at a flow rate of 1.5 ml/min. Quantitation was achieved with ultraviolet detection at 235 nm over concentration ranges of 0.2-4 and 0.1-2 μg/ml; mean accuracies were 101.16±0.59 and 99.79±0.56% for fluoxetine HCL and olanzapine, respectively.

6. Sandeep B. Patil et al.,(2009)[10] Carried out preparation of Olanzapine, quick dispersing tablets by direct compression method. Effect of super disintegrant cross povidone on wetting time, disintegration time, and drug content and in vitro release have been studied. A 32 factorial Design was employed informulating a quick dispersible tablet. The selected independent variables crospovidone and hydroxyl propylcellulose showed significant effect on dependent variables i.e.
Disintegration time and percent drug dissolved. Disintegration time and percent drug dissolved decreased with increase in the level of cross povidone. The similarity factor *f*2 was found to be 72.68 for the developed formulation indicating the release was similar to that of the marketed formulation.

7. **Prameela rani.et al., (2009)[13]** Carried out A reverse phase HPLC method is developed for the determination of olanzapine in pharmaceutical dosage forms. Chromatography was carried out on an inertsil C18 column using a mixture of ammonium phosphate buffer and methanol (70:30 v/v) as the mobile phase at a flow rate of 1 ml/min. Detection was carried out at 220 nm .The retention time of the drug was 3.447min. The method produced linear responses in the concentration range of 2 to 10µg/ml of olanzapine. The method was found to be applicable for determination of the drug in tablets.

8. **M. Jagadeeswaran et al., (2009)[21]** Developed A simple, accurate, low cost and specific HPTLC method for estimation of Fluoxetine in capsule has been developed. It was performed on Silica gel G60 F254 aluminum foil using acetonitrile: chloroform in the ratio of 1:9 as mobile phase. The mobile phase having chamber was saturated for 15 minutes at room temperature. The Rf value of Fluoxetine was found to be 0.4. The plate was scanned and quantified at 254 nm. The calibration curve response was observed between 4-20 µg. The linear regression data showed good linear relationship of r = 0.9986. The percent recovery was found to be 100.0 ± 0.01.The developed method was validated for its accuracy and precision with suitable parameters.

9. **Deepa Sharma et al.,(2010)[7]** carried out Simultaneous estimation of risperidone, olanzapine and quetiapine and their degradation products by HPLC . A rapid, specific reversed phase HPLC method has been developed for simultaneous determination of risperidone, olanzapine and quetiapine. Drugs were subjected to stress conditions such as acidic, alkaline and oxidative Hydrolysis. Chromatographic separation of these pure drugs was carried out on Luna C18 (250*4.6, 56m) with a 50:50 (v:v)mixture of 20mM ammonium acetate and acetonitrile as Mobile phase. The flow rate was 1.0 mL min-1 and the analysis was monitored at 235 nm by UV detection. The system and method precision was found to be less than 1%. The assay results were linear from 35 to 65 µg mL=1 for risperidone ($R2 > 0.991$), olanzapine ($R2 > 0.992$) and quetiapine ($R2 > 0.999$). Method validated showed it to be specific, precise, robust and linear over the range of analysis.

10. **Zahid Zaheer et al.,(2010)[16]** Developed A simple, accurate, low cost and specific HPTLC method for estimation of Fluoxetine hydrochloride in capsule has been developed. It was performed on Silica gel G_{60} F_{254} aluminum foil using Acetone: Methanol in the ratio of 5:4 as mobile phase. The mobile phase containing chamber was saturated for 10 minutes at room temperature. The Rf value of Fluoxetine was found to be 0.12. The plate was scanned and quantified at 226 nm.

11. **Moinuddin r syed et al.,(2010)**[18] Developed A simple and reproducible method was developed for the assay of paroxetine in tablets. The excipients in the commercial tablet preparation did not interfere with the assay. Beer's law is obeyed in the range 2.0 - 10.0 µg.mL–1 at .max 294 nm. The molar absorptivity was calculated. Six triplicate analyses of solutions containing six different concentrations of the examined drug were carried out and gave a mean correlation coefficient 0.999.The proposed method was applied to the determination of the examined drug in market tablet and the results demonstrated that the method isequally accurate, precise and reproducible as the official methods.

12. **Poornachander Thatipalli et al.,(2010)**[11] Carried out HPLC analysis of olanzapine , a known anti-psychotic drug, showed impurity peaks ranging from 0.05 to 0.15 % during process development. These samples were analyzed by LCMS and the peaks were identified at *m/z* 230, 341, 511, 326, 361 and 329. All six impurities were synthesized individually and characterized based on their spectral data (IR, NMR and Mass).

13. **Vijaykumar K. Parmar et al., (2011)**[4] Developed spectrophotometric determination of fluoxetine hydrochloride and olanzapine in tablets . Fluoxetine hydrochloride (FH) in combination with Olanzapine (OZ) is used in treatment of depressive episodes associated with bipolar disorder. The first derivative spectrophotometric method for simultaneous determination of fluoxetine hydrochloride (FH) and olanzapine (OZ) from two component tablet dosage form has been developed and validated. Chromatographic methods viz. HPLC and HPTLC methods were reported in the literature for the simultaneous determination of FH and OZ from combined dosage forms. In the present investigation an attempt has been made to develop accurate, reproducible, rapid and cost-effective method for simultaneous determination of FH and OZ in pharmaceutical formulation. The wavelengths selected for determination of FH and OZ is 235.5nm and 296nm, respectively.

14. **Eirik Kjelby et al.,(2011)**[8] Carried out Efficacy studies indicate anti-depressive effects of at least some second generation antipsychotics(SGAs). The Bergen Psychosis Project (BPP) is a 24-month, pragmatic, industry-independent, randomized, head-to head comparison of olanzapine, quetiapine, risperidone and

ziprasidone in patients acutely admitted with psychosis. The aim of the study is to investigate whether differential anti-depressive effectiveness exists among SGAs in a clinically relevant sample of patients acutely admitted with psychosis.

15. **R. Satheesh Kumar et al.,(2011)[19]** Developed Spectrophotometric method is a simple technique for determination of fluoxetine in bulk drug and its pharmaceutical formulation. Fluoxetine is mainly used as antidepressant. Fluoxetine shows maximum absorbance at 261nm in Distilled water. Beer's law obeyed concentration range of 400mcg/ml-800mcg/ml with the correlation coefficient of 0.9994. The results of analysis were validated statically and by recovery studies and found to be satisfactory. The proposed method was extended to pharmaceutical formulations and there was no interference of additives and excipients.

16. **Jordana S. Bueno et al.,(2011)[20]** Developed the enantio selective analysis of fluoxetine and norfluoxetine in plasma samples was performed by the protein precipitation method and high performance liquid chromatography with fluorescence detection (PP/LC-FD). Different precipitating agents - organic solvents, acids, and salts - in several proportions were available. The Bradford colorimetric method employed for evaluation of the efficiency of protein precipitation, has shown that for the sake of simplicity and percentage of protein precipitation (99.7%), acetonitrile was most effective when added at a ratio of 3:1 (acetonitrile/plasma, v/v). The quantification limit of the PP/LC-FD method was 30 ng mL−1 for the four enantiomers.

3.1 Aim and objective

Aim:

The aim is to develop and validate a new, simple, accurate, precise and rapid method for simultaneous Olanzapine and Fluoxetine Hcl by RP-HPLC.s

Objective:

The pharmaceutical companies are manufacturing multiple drug formulations to meet the market demand and to treat the patients who are unresponsive to single drug formulations. Very few methods are available for simultaneous estimation of multiple drug formulations.

Standard analytical procedure for simultaneous estimation of multiple drug formulations may not be available in pharmacopoeias; hence it is essential to develop newer analytical methods which are accurate, precise, specific, linear, simple and rapid.

From the literature survey it has been concluded that several method were reported for estimation of Olanzapine and Fluoxetine Hcl individually and few methods are available for simultaneous estimation of these drugs but none of the reported analytical methods describe a method for the simultaneous determination of Olanzapine and Fluoxetine Hcl having less run time with good resolution.

Hence, the present work was thought to develop a precise, accurate, simple and reliable, less time consuming method for simultaneous estimation of Olanzapine and Fluoxetine Hcl by RP-HPLC.

PLAN OF WORK

In present study, an attempt has been made to developed RP-HPLC method for the simultaneous estimation of Olanzapine and Fluoxetine HCl in their combined dosage form.

- Literature survey
- Selection of λmax
- Selection of Mobile Phase
- Selection of Column
- Method development & Optimization
- Method of Validation

3.3 MATERIALS

3.3.1 Chemicals used:

S.No	Chemicals	Grade	Manufacturer/Supplier
1.	Water	HPLC	Microlabs
2.	Methanol	HPLC	Merck
3.	Acetonitrile	HPLC	Merck
4.	Potassiumdihydrogen phosphate	AR	Merck
5.	Sodium dihydrogen phosphate	AR	Merck
6.	0.1M Sodium hydroxide	AR	Merck
7.	Hydrochloric acid	AR	Merck
8.	Triethylamine	AR	Spectrochem
9.	Ortho phosphoric acid	AR	Merck

3.3.2 Equipment used:

S.No	Name	Model	Manufacturer/Supplier
1.	Analytical balance	Unibloc	Shimandzu, Libror
2.	pH meter	Eutech	Shimandzu.
3.	HPLC	LC-2010	Shimandzu Corporation, Japan
4.	UV	UV-2550	Shimandzu Corporation

Drug sample

Olanzapine, Fluoxetine Hcl sample obtained from Chandra labs Pvt., Ltd., Hyderabad.

Formulation used

Prandimet tablets containing 10 mg Olanzapine and Fluoxetine Hcl 20 mg were proceed from Chandra labs Pvt., Ltd., Hyderabad.

Solubility

Solubility of drugs was observed by dissolving it in different solvents and it was found that drugs having good solubility in following solvents.

Table No 4:

S.no	Solvent	Solubility	
		Olanzapine	Fluoxetine HC
1.	Water	+	+
2.	Acetonitrile	+	+
3.	0.1N NaOH	+	+
4.	0.1N HCl	+	+

3.4 METHOD DEVELOPMENT

3.4.1 TRAIL-1

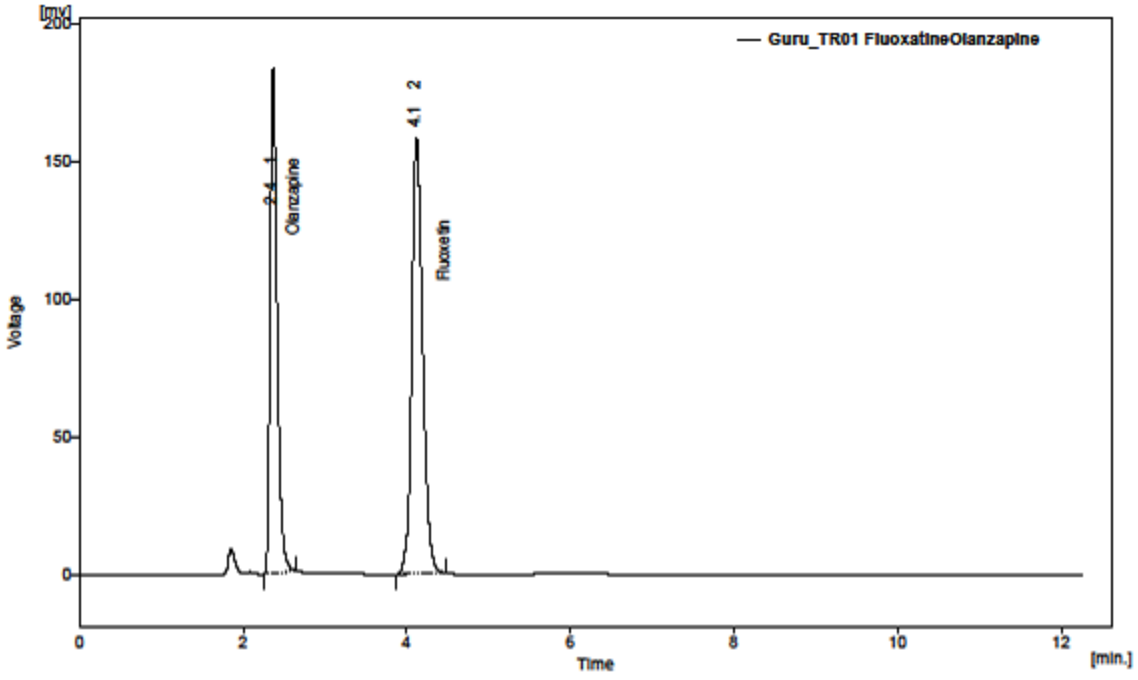
1. **Preparation of Buffer:**

   Take 295mg potassium dihydrogen phosphate of 0.02 M was dissolved in 100ml of water and adjust the pH-3 using diluted O-phosphoric acid.

2. **Preparation of mobile phase:**

   Filtered and degassed mixture of acetonitrile: buffer in the ratio of 50:50 and filter through 0.45 micron membrane filter.

Fig: 1 Olanzapine and Fluoxetine HCl (Acetonitrile: Potassium Di Hydrogen Phosphate Buffer (50:50 V/ V))



3.4.2 TRAIL-2

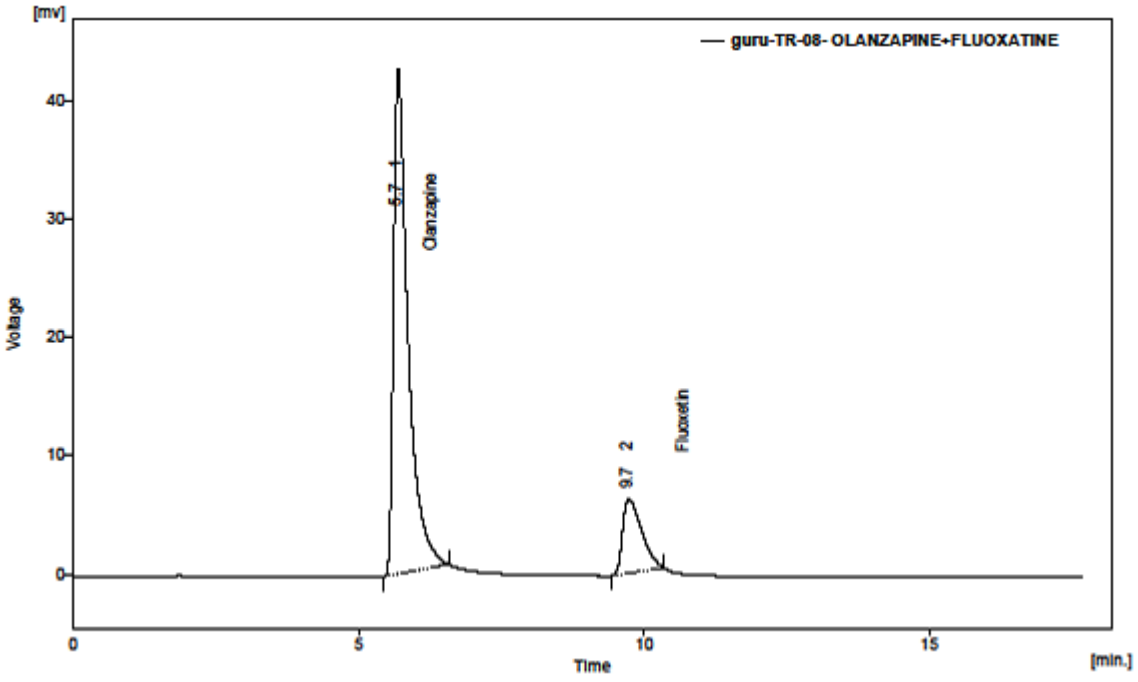
1. Preparation of Buffer:

Take 295 mg of 0.02 M potassium dihydrogen phosphate was dissolved in 100ml of water and adjust the pH-4.5 using diluted O-phosphoric acid.

2. **Preparation of mobile phase:**

Filtered and degassed mixture of acetonitrile: buffer in the ratio of 40:60 and filter through 0.45 micron membrane filter.

Fig: 2 Olanzapine and Fluoxetine HCl (Acetonitrile: Potassium Di Hydrogen Phosphate Buffer (40:60 V/ V))



3.4.3 TRAIL-3

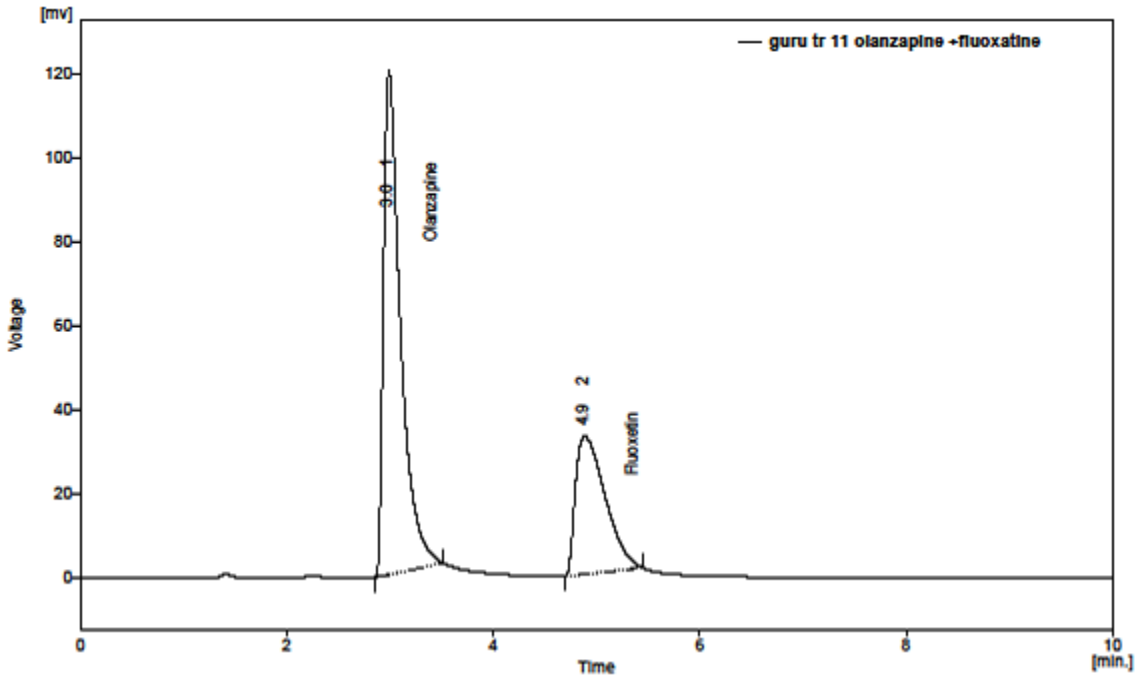
1. Preparation of Buffer:

Take 54 mg of 0.03 M of dipotassium hydrogen phosphate and 295 mg of 0. 02 M of potassium di hydrogen phosphate was dissolved in 1000ml of water and adjust the pH-3 using diluted O-phosphoric acid.

2. Preparation of mobile phase:

Filtered and degassed mixture of acetonitrile: buffer in the ratio of 40:60 and filter through 0.45 micron membrane filter.

Fig: 3 Olanzapine and Fluoxetine HCl (Acetonitrile: Mixed Phosphate Buffer (40:60 V/ V))



3.4.4 TRAIL-4

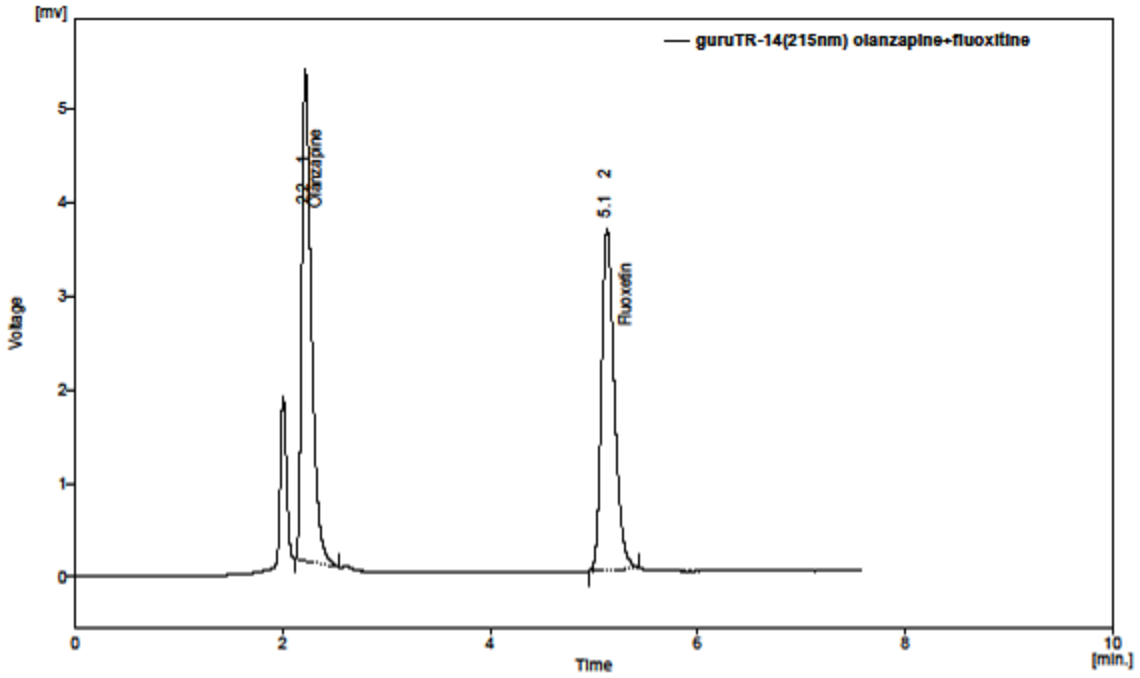
1. Preparation of Buffer:

Take 54 mg of 0.03 M of dipotassium hydrogen phosphate and 295 mg of 0.02 M potassium dihydrogen phosphate was dissolved in 1000ml of water and adjust the pH-6 using diluted O-phosphoric acid.

2. Preparation of mobile phase:

Filtered and degassed mixture of acetonitrile: buffer in the ratio of 50:50 and Filter through 0.45 micron membrane filter.

Fig: 4 Olanzapine and Fluoxetine HCl (Acetonitrile: Mixed Phosphate Buffer (50:50 V/ V))



3.4.5 TRAIL-5

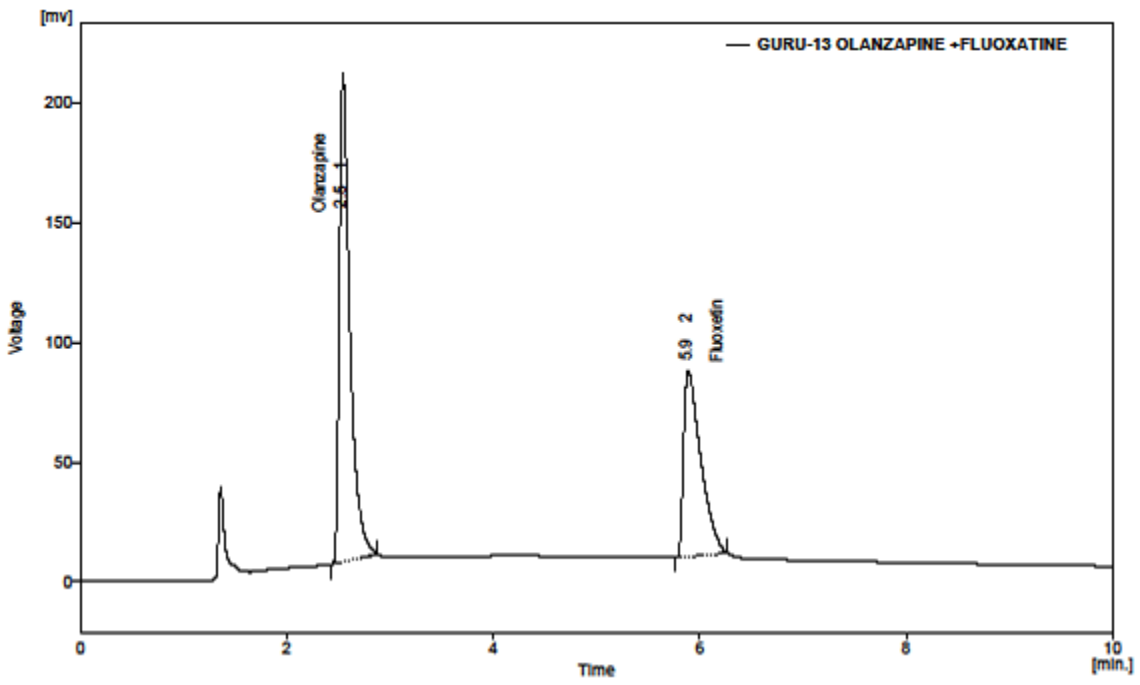
1. Preparation of Buffer:

Take 54 mg of 0.03 M of dipotassium hydrogen phosphate and 295 mg of 0.02M potassium dihydrogen orthophosphate was dissolved in 1000ml of water and adjust the pH-3 using diluted O-phosphoric acid.

2. Preparation of mobile phase:

Filtered and degassed mixture of acetonitrile: buffer in the ratio of 45:55 and filter through 0.45 micron membrane filter.

Fig : 5 Olanzapine and Fluoxetine HCl (Acetonitrile: Mixed Phosphate Buffer (45:55 V/ V))



3.4.6 TRAIL-6

1. Preparation of Buffer:

Take 54 mg of 0.03 M of dipotassium hydrogen phosphate and 295 mg of 0.02 M potassium dihydrogen phosphate was dissolved in 100ml of water and adjust the pH-3 using diluted O-phosphoric acid.

2. Preparation of mobile phase:

Filtered and degassed mixture of acetonitrile: buffer in the ratio of 40:55 and Filter through 0.45 micron membrane filter.

TABLE NO 2 :

Trial	Mobile	Column	Result	Wavelength	P^H	FLOW
1	ACN:KH2PO4 (50:50)	WATERS SYMMETRY C18(250x4.6)	Asymmetry was not good	235	5.5	1ml/1min
2	ACN:KH2PO4 (40:60)	BDS hypersil C18(250x4.6)	Less retention time	235	4.5	1ml/1min
3	ACN:KH2PO4 and K2HPO4 (40:60)	WATERS C18(250x4.6)	Asymmetry was not good	235	4.5	1ml/1min
4	ACN:KH2PO4 and K2HPO4 (50:50)	WATERS SYMMETRY C18(250x4.6)	Asymmetry was not good	215	6	1.2ml/1min
5	ACN:KH2PO4 and K2HPO4 (45:55)	WATERS SYMMETRY C18(250x4.6)	Optimized chromatogram	235	3	1ml/1min
6	ACN:KH2PO4 and K2HPO4 (45:55)	WATERS SYMMETRY C18(250x4.6)	Good result	235	3	1ml/1min

Initialization of the instrument

Initially, the column was placed on the instrument and switch on the instruments and washed with acetonitrile: water (20:80) for 30 min. then the system was made to run with the mobile phase for 30 min for column saturation.

Standard preparation of Olanzapine and Fluoxetine HCl

Standard-A: Accurately weighed quantity of 10mg Olanzapine was transferred in to a 100 ml volumetric flask and made up to the volume with diluents. From this 5 ml was pipetted out in to a 50 ml volumetric flask and made up to the volume with same diluents.

Standard-B: Accurately weighed quantity of 20 mg Fluoxetine HCl was transferred in to a 100 ml volumetric flask and made up to the volume with diluents. From this 5 ml was pipetted out in to a 50 ml volumetric flask and made up to the volume with same diluents

TABLE NO 3: CHROMATOGRAPHIC CONDITIONS

Mode of operation	Isocratic
Parameters	Description
Diluents	Water
Column	C18, 250x4.6mm,5µ SS column
Mobile phase	Acetonitrile: mixed buffer (45:55)
Flow rate	1.0 ml/min
Detection of Olanzapine and Fluoxetine HCl	235 nm
Temperature	25^0 C
Injection Volume	20 µl
Run time	20 min
Detector	UV detector

3.4.7 TABLE NO : 1 OPTIMIZED METHOD PARAMETERS

PARAMETERS	CONDITIONS
Column (Stationary Phase)	WATERS C18 Symmetry (4.6 x 250mm, 5 µm
Mobile Phase	Mixed Buffer: Acetonitrile (55:45)
Flow rate (ml/min)	1ml/mm.
Run time (min)	20
Column temperature(°C)	25^0 C
Volume of injection loop (µl)	20µl
Detection wavelength (nm)	235
Drug RT (min)	2.2, 5.05

3.5 VALIDATION PARAMETERS

Since the HPLC method has been developed, validation of method using various parameters was performed to ensure that the performance characteristic of the method meets the requirements for the intended analytical applications.

I. SYSTEM SUITABILITY :

Preparation of standard solution:

Accurately weighed 10 mg olanzapine and 20 mg fluoxetine in 50 ml volumetric flask dilute to Volume with mobile phase volumetric flask dilute to volume with mobile phase.

Preparation of test solution:

About 0.8500 g of the sample was taken in 100 ml volumetric flask and added 15 ml of mobile phase and sonicated for 15 min to dissolve the content and made up to the volume. From this 5 ml was pipette out into 100 ml volumetric flask and made up to the volume with same mobile phase. Filter the content by using 0.45µ membrane filter by applying vacuum.

II. SPECIFICITY

Specificity is the ability to assess unequivocally an analyte in the presence of components that may be expected to be present. For the simultaneous determination of olanzapine and fluoxetine HCl the specificity requires that the method should not be affected by the presence of other components. Usually the specificity would be performed by allowing the sample under stressed conditions.

i) Heating

For the specificity study 1 mL from the stock solution should be taken in a 10 mL flask, make up to the volume with the mobile phase. The solution should be heated at 40 ^{o}C for a period of 30 min. Observe for any degradation occurs or not.

ii) Treating with Acids

Take 1 mL from the stock solution into a 10 mL flask. To that flask add 1 mL of 0.1M hydrochloric acid. Observe for any change took place in the retention of the peak.

iii) Treating with Base

Take 1 mL from stocks solution into a 10 mL flask, and add 1 mL of 0.1 M sodium hydroxide. Observe for any degradents.

III. LINEARITY:

Preparation of standard stock solution

Accurately weighed 10 mg olanzapine and 20 mg fluoxetine in 50 ml volumetric flask dilute to volume with mobile phase volumetric flask dilute to volume with mobile phase.

Preparation of linearity solution-I: Transfer 1ml from stock solution to 10 ml with mobile phase(the solution becomes 2mcg of olanzapine and 4mcg of fluoxetine)

Preparation of linearity solution-II: Transfer 2ml from stock solution to 10 ml with mobile phase(the solutionbecomes 4mcg of olanzapine and 8mcg of fluoxetine)

Preparation of linearity solution-III: Transfer 3ml from stock solution to 10 ml with mobile phase(the solution becomes 6mcg of olanzapine and 12mcg of fluoxetine)

Preparation of linearity solution-IV: Transfer 4ml from stock solution to 10 ml with mobile phase(the solution becomes 8mcg of olanzapine and 16mcg of fluoxetine)

Preparation of linearity solution-V: Transfer 5ml from stock solution to 10 ml with mobile phase(the solution becomes 10mcg of olanzapine and 20mcg of fluoxetine)

Preparation of linearity solution-VI: Transfer 6ml from stock solution to 10 ml with mobile phase(the solution becomes 12mcg of olanzapine and 24mcg of fluoxetine)

Determination: The linearity of the analytical method is determined by mathematical treatment of test results obtained by analysis of samples with analyte concentrations across the claimed range. Area is plotted graphically as a function of analyte concentration. Percentage curve fittings are calculated.

Acceptance criteria: the correlation coefficient and regression coefficient shall be not less than 0.99 for Olanzapine and Fluoxetine HCl.

IV. SYSTEM PRECISION:

Preparation of Stock solution:

Weigh 10 mg Olanzapine and 20 mg Fluoxetine in 50 ml Volumetric Flask Dilute To Volume with Mobile Phase

Dilution:

Transfer 5ml from stock solution to 100 ml with mobile phase (the solution becomes 10mcg of olanzapine and 20mcg of fluoxetine)

V. ACCURACY:

Preparation of Stock Solution:

Weigh 10 mg olanzapine and 20 mg fluoxetine in 50 ml volumetric flask dilute to volume with mobile phase.

Preparation of Spiking standard:

Transfer 5 ml from stock solution to 100 ml with mobile phase.

Preparation of Accuracy solution 1: Transfer 4ml from stock solution to 100 ml with mobile phase (The solution becomes 8mcg of olanzapine and 16mcg of fluoxetine) and add 1 ml of spiking standard.

Preparation of Accuracy solution 2: Transfer 5ml from stock solution to 100 ml with mobile phase (The solution becomes 10mcg of olanzapine and 20mcg of fluoxetine) and add 1 ml of spiking standard.

Preparation of Accuracy solution 3: Transfer 6ml from stock solution to 100 ml with mobile phase (The solution becomes 12mcg of olanzapine and 24mcg of fluoxetine) and add 1 ml of spiking standard.

VI. METHOD PRECISION:

Stock solution:

Weigh 200 mg of the sample In 50 ml Volumetric Flask, Dilute to Volume with Mobile Phase

Dilution:

Transfer 5ml from stock solution to 100 ml with mobile phase (the solution becomes 10mcg of olanzapine and 20mcg of fluoxetine)

VII. ASSAY:

Preparation of Stock Solution:

Weigh 10 mg olanzapine and 20 mg fluoxetine in 50 ml volumetric flask dilute to volume with mobile phase.

Preparation of Standard solution:

Transfer 5ml from stock solution to 100 ml with mobile phase (The solution becomes 10mcg of olanzapine and 20mcg of fluoxetine)

Preparation of Sample solution:

Weigh 10 tablets, calculate the average weight, powdered, weigh 200.6mg in 50 ml with mobile phase, transfer 5 ml to 100 ml with mobile phase.

VIII. LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ):

The LOD and LOQ of the drug were derived by visually or calculating the signal-noise ratio. In this method the LOD and LOQ of the drug were calculated by following equation.

$$LOD = \frac{3.3 \times \text{Standard deviation}}{\text{Slope}}$$

$$LOQ = \frac{10.3 \times \text{Standard deviation}}{\text{Slope}}$$

IX.RUGGEDNESS:

Ruggedness study was carried out by repeating the complete experiment with different analysts, on different days in same laboratory as per the following preparation.

Blank solution: Purity water was used as diluents.

Stock solution:

Weigh 10 mg Olanzapine and 20 mg Fluoxetine In 50 ml Volumetric Flask Dilute To Volume With Mobile Phase

Dilution:

Transfer 5ml from stock solution to 100 ml with mobile phase (the solution becomes 10mcg of olanzapine and 20mcg of fluoxetine)

Determination: the Ruggedness of the analytical method and instrument is determined by assaying sufficient number of samples and relative standard deviation is calculated.

Acceptance criteria: the relative standard deviation should not be less than 2 %.

X. ROBUSTNESS:

Stock solution:

Weigh 10 mg Olanzapine and 20 mg Fluoxetine In 50 ml Volumetric Flask Dilute To Volume With Mobile Phase

Dilution:

Transfer 5ml from stock solution to 100 ml with mobile phase (the solution becomes 10mcg of olanzapine and 20mcg of fluoxetine)

S. No	Chromatographic condition	Low	High
1.	Flow rate	0.9 ml	1.1 ml
2.	Wavelength	233 nm	237nm

Determination: the robustness of an analytical method is to determine by analysis of aliquots from homogenous lots by differing physical parameters that may differ but are still within the specified parameters of the assay.

Acceptance criteria: the percentage assay of drugs should be within the limit of 90- 110%.

IV. RESULTS AND DISCUSSION

TRAIL-1

System suitability results

Olanzapine and Fluoxetine hydrochloride

1.) Tailing factor obtained from trail- 1 was 1.700 and 1.412

2.) Theoretical plates obtained from trail-1 was 3562 and 4798

3.) Resolution obtained from trail-1 was 8.843

4.) Retention time obtained from trail-1 was 2.367 and 4.120

TRAIL-2

System suitability results

Olanzapine and Fluoxetine hydrochloride

1.) Tailing factor obtained from trail- 1 was 2.909 and 2.458

2.) Theoretical plates obtained from trail-1 was 2795 and 3635

3.) Resolution obtained from trail-1 was 7.513

4.) Retention time obtained from trail-1 was 5.690 and 9.733

TRAIL-3

System suitability results

Olanzapine and Fluoxetine hydrochloride

1.) Tailing factor obtained from trail- 1 was 2.893 and 2.800

2.) Theoretical plates obtained from trail-1 was 1714 and 1215

3.) Resolution obtained from trail-1 was 4.464

4.) Retention time obtained from trail-1 was 2.990 and 4.887

TRAIL-4

System suitability results

Olanzapine and Fluoxetine hydrochloride

1.) Tailing factor obtained from trail- 1 was 1.950 and 1.533

2.) Theoretical plates obtained from trail-1 was 2730 and 7796

3.) Resolution obtained from trail-1 was 14.454

4.) Retention time obtained from trail-1 was 2.220 and 5.127

TRAIL-5

System suitability results

Olanzapine and Fluoxetine hydrochloride

1.) Tailing factor obtained from trail- 1 was 2.579 and 3.417

2.) Theoretical plates obtained from trail-1 was 3150 and 5718

3.) Resolution obtained from trail-1 was 13.581

4.) Retention time obtained from trail-1 was 2.543 and 5.890

TRAIL-6

System suitability results

Olanzapine and Fluoxetine hydrochloride

1.) Tailing factor obtained from trail- 1 was 1.5 and 1.4

2.) Theoretical plates obtained from trail-1 was 2500 and 7020

3.) Retention time obtained from trail-1 was 2.2 and 5.05

TRAILS RESULT

On the evaluation of above system suitability results,

- In trail-II and trail-III the tailing factor is more than 2 so it is not satisfactory

- In trail III theoretical plates should to be increased.
- In trail-IV and trail-V the resolution should to be reduced.
- System suitability parameters of trail-VI were within the satisfactory limits.

Hence trail I,II, III,IV,V shows variation in system suitability results and affected the method significantly .Trail-VI shows good system suitability results and also within in the limit so the trail – VI was adopted.

VALIDATION RESULTS

SYSTEM SUITABILITY

Table no 5: System suitability results for olanzapine:

S.No	Parameter	Olenzapine
1	RT(min)	2.2
2	Tailing Factor	1.5
3	No.of theoretical plates	2557.000

Table no 6: System suitability results for Fluoxetine HCl:

S.No	Parameter	Fluoxetine HCl
1	RT(min)	5.05
2	Tailing Factor	1.4
3	No.of theoretical plates	7051.000

Result:

On the evaluation of above results it was found that all the system suitability parameters were within the satisfactory limit.

SPECIFICITY

Diluents, standard preparation and assay were prepared as per the method and the solutions were injected into the chromatograph and the chromatograms recorded. The retention time given in the following table,

Table no-7: Specificity results for Olanzapine and Fluoxetine hydrochloride

S.NO	SOLUTION	RETENTION TIME(min)
1.	Olanzapine Standard preparation	2.23
2.	Olanzapine assay preparation	2.01
3.	Fluoxetine Hcl standard preparation	5.05
4.	Fluoxetine Hcl assay preparation	5.02

Table no-8 Specificity results for Olanzapine and Fluoxetine hydrochloride under stress conditions

S.NO	STRESS CONDITIONS	OBSERVED RESULT
1.	Heated on water bath	No degradation occurred
2.	Treated with acids	No change in retention of the peak
3.	Treated with base	No degradents formed

Result:

i.) No peaks should be detected at the retention time of Olanzapine and Fluoxetine hydrochloride in the chromatograms of diluents preparation

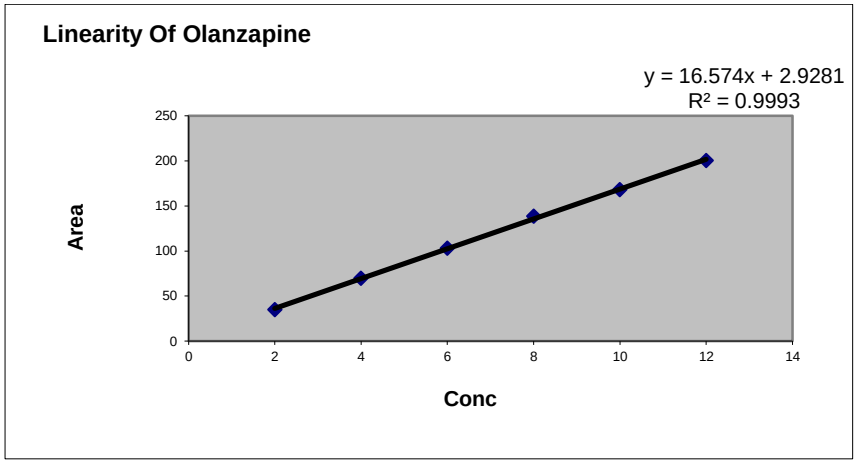
ii.) From the stress conditions performed, various degradation products were formed and there was no change in the detection of the analyte in the presence of other components.

LINEARITY

TABLE NO 9: Linearity Results: (for olanzapine)

S.No	Linearity Level	Concentration	Area
1	I	2 mcg	34.565
2	II	4 mcg	69.423
3	III	6 mcg	102.909
4	IV	8 mcg	138.428
5	V	10 mcg	167.979
6	VI	12 mcg	200.359
Correlation Coefficient			0.999

Acceptance Criteria: Correlation coefficient should be not less than 0.9

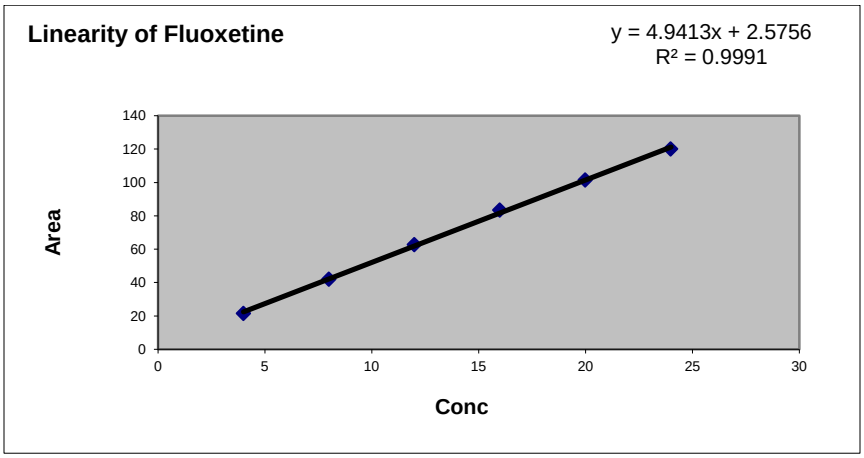


CALIBRATION PLOT FOR OLANZAPINE

TABLE NO 10 Linearity Results: (for fluoxetine)

S.No	Linearity Level	Concentration	Area
1	I	4 mcg	21.437
2	II	8 mcg	41.825
3	III	12 mcg	62.651
4	IV	16 mcg	83.335
5	V	20 mcg	101.31
6	VI	24 mcg	119.966
Correlation Coefficient			0.999

Acceptance Criteria: Correlation coefficient should be not less than 0.9



CALIBRATION PLOT FOR FLUOXETINE

DISCUSSION

On evaluation of above results % RSD values are within the limit hence the curve shows linearity.

SYSTEM PRECISION

TABLE NO 11- The results are summarized (olanzapine)

Injection	**Area**
Injection-1	175.486
Injection-2	172.348
Injection-3	173.659
Injection-4	172.838
Injection-5	172.537
Average	173.3736
Standard Deviation	1.282801
%RSD	**0.74**

TABLE NO 12 - The results are summarized (fluoxetine)

Injection	**Area**
Injection-1	104
Injection-2	104.755
Injection-3	103.428
Injection-4	104.507
Injection-5	104.583
Average	104.2546
Standard Deviation	0.540842
RSD	**0.52**

Acceptance Criteria:

The % RSD for the area of five standard injections results should not be more than 2%

DISCUSSION:

On the evaluation above results % RSD values are within the limit hence the method is precise.

ACCURACY

TABLE NO 13 - The accuracy results for olanzapine

%Concentration (at specification Level)	Area	Amount Added (mcg)	Amount Found (mcg)	% Recovery	Mean Recovery
80%	154.4297	9	8.93	99.26%	99.44%
100%	189.5143	11	10.96	99.66%	
120%	223.4167	13	12.92	99.41%	

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.0%

TABLE NO 14 – The accuracy results for fluoxetine

%Concentration (at specification Level)	Area	Amount Added (mcg)	Amount Found (mcg)	% Recovery	Mean Recovery
80%	92.97033	18	17.88	99.34%	99.59%
100%	113.7897	22	21.89	99.48%	
120%	135.1153	26	25.99	99.95%	

Acceptance Criteria:

- The % Recovery for each level should be between 98.0 to 102.0

DISCUSSITON:

On the evaluation above results % recovery of the drug shows 99.5 % hence the method is found to be accurate.

METHOD PRECISION

TABLE NO 15: Method precision results (Olanzapine):

S.No.	Rt	Area
1	2.21	171.772
2	2.19	173.657
3	2.217	173.768
4	2.2	174.41
5	2.207	172.398
avg	2.2048	173.201
stdev	0.010281	1.081429
%RSD	**0.47**	**0.62**

TABLE NO 16: Method precision results (Fluoxetine):

S.No.	Rt	Area
1	5.077	104.183
2	5.053	104.585
3	5.097	105.061
4	5.053	104.819
5	5.073	104.958
avg	5.0706	104.7212
stdev	0.018461	0.349754
%RSD	**0.36**	**0.33**

Acceptance Criteria:

The % RSD for the area of five standard injections results should not be more than 2%

DISCUSSION:

On the evaluation above results % RSD values are within the limit hence the method is precise.

ASSAY

Calculation for olanzapine:

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg.Wt}}{\text{Label claim}} \times 100$$

Where:

AT = average area counts of sample preparation.

As= average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = Label claim of olanzapine mg/ml.

173.404 0.01 10 99.7 0.2

---------- x ---------- **x**----------x----------x---------- =x100=100.02

172.8567 10 0.2 100 0.01

Calculation for fluoxetine hydrochloride

Assay%=

AT WS DT P Avg.Wt

----------X----------X----------X----------X----------x100

AS DS WT 100 Label claim

Where:

AT = average area counts of sample preparation.

As= average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = LABEL CLAIM OF fluoxetine hydrochloride **mg/ml.**

103.681 0.02 20 99.8 0.2

----------x----------x----------x----------x---------- x 100=99.66

104.0327 20 0.2 100 0.02

RESULT:

1.) The percentage purity of olanzapine=100.22%

2.) The percentage purity of fluoxetine hydrochloride=99.66

LIMIT OF DETECTION: (for olanzapine)

$$LOD = 3.3 \times \frac{\text{Std deviation}}{\text{Slope}}$$

$$= 3.3 \times \frac{0.008019}{0.999}$$

$$=0.29040\ \mu g/ml$$

LIMIT OF QUANTIFICATION: (for olanzapine)

$$LOD = 10.3 \times \frac{\text{Std deviation}}{\text{Slope}}$$

$$= 10.3 \times \frac{0.008019}{0.999}$$

$$=0.90600\ \mu g/ml$$

LIMIT OF DETECTION: (for Fluoxetine)

$$LOD = 3.3 \times \frac{\text{Std deviation}}{\text{Slope}}$$

$$= 3.3 \times \frac{0.02045}{0.999}$$

$$= 0.36120\ \mu g/ml$$

LIMIT OF QUANTIFICATION: (for Fluoxetine)

$$LOD = 10.3 \times \frac{\text{Std deviation}}{\text{Slope}}$$

$$= 10.3 \times \frac{0.02045}{0.999}$$

$$= 1.12720\ \mu g/ml$$

RUGGEDNESS

Table no -17 intermediate precision (ruggedness) for Olanzapine:

Injections	**Area**	
	Analyst-1	**Analyst-2**
1	174.236	175.224
2	172.238	172.344
3	173.244	174.432
4	173.322	171.238
5	174.414	173.224
6	172.314	173.382
Avg	173.2947	173.3073
Std dev	0.918758	1.425564
%RSD	**0.52**	**0.82**

Table no -18 intermediate precision (ruggedness) for fluoxetine HCl :

Injections	**Area**	
	Analyst-1	**Analyst-2**
1	105.715	106.734
2	104.722	105.735
3	105.744	106.765
4	103.755	106.788
5	105.711	105.734
6	104.760	105.797
Avg	105.0678	106.089
Std dev	0.80344	1.336743
%RSD	**0.76**	**0.84**

DISCUSSION:

The %RSD is less than 2% for the results of two analyst indicating the ruggedness of the method.

ROBUSTNESS:

The robustness of the method established by making minor variations in the method parameters like, change in flow rate by ± 10 % of actual flow rate .

Table no-19 results for Olanzapine

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	3191	1.544
2	1.1	7037	1.446

Table no-20 Results for Fluoxetine hydrochloride:

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	7512	1.431
3	1.1	2772	1.518

Table no-21 results for Olanzapine

S.No	wavelength (nm)	System Suitability Results	
		USP Plate Count	USP Tailing
1	233	2878	1.512
2	237	7037	1.446

Table no-22 results for Fluoxetine hydrochloride:

S.No	wavelength (nm)	System Suitability Results	
		USP Plate Count	USP Tailing
1	233	3022	1.512
3	237	7151	1.421

Result:

The flow rate was varied at 0.9 ml/min to 1.1ml/min

On evaluation of the above results, it can be concluded that the variation in flow rate and wavelength not affected the method significantly. Hence it indicates that the method is not robust even by change in the flow rate and wavelength ±10%.

V. SUMMARY AND CONCLUSION

An attempt has been made to develop the RP-HPLC method for simultaneous estimation of Olanzapine and Fluoxetine Hcl in combined dosage form. As the literature survey revealed that few methods were available for their simultaneous estimation, but there is a need of a simple, economical and proper method for estimation of above combination in combined dosage form.

Water's- HPLC with spinchrom 21cfr software, UV detector and with symmetry C_{18} Column (250mm X 4.6 mm, 5µ), an injection volume of 20µl is injected and eluted with the mobile phase of mixed buffer (0.02M Potassium dihydrogen phosphate +0.03 M diPotassium hydrogen phosphate):Acetonitrile (55: 45), which was pumped at a flow rate of 1ml/min and detected by UV detector at 235nm. The peaks of Olanzapine and Fluoxetine Hcl were found well separated at, 2.2 and 5.1 respectively.

The specificity performed shows various degradation products and there is no change in the detection of the analyte in the presence of other components.

The assay study was found that the formulation contains 100.2% of Olanzapine and 99.6%.of Fluoxetine Hcl.

The system suitability studies was found that all the system suitability parameters were within the acceptance criteria.

The linearity was found that the drug obeys linearity within the concentration range of 2 - 12µg/ml for Olanzapine, 4-24µg/ml for Fluoxetine Hcl.

The results shown in accuracy was found that the percentage recovery values of pure drug from the preanalyzed solutions of formulations were in between 99.4% for Olanzapine , 99.5% for Fluoxetine Hcl, which indicates that the method was accurate.

The result shown in precision was found that % RSD is less than 2%; which indicates that the proposed method has good reproducibility.

The results shown in Robustness were found that there is little change in the results with the change in the parameters like flow rate and wavelength, indicating the robustness of the method.

From the results shown in the ruggedness, the %RSD is less than 2% for the results of two analyst indicating the ruggedness of the method.

The developed chromatographic method for the determination of Olanzapine And Fluoxetine Hcl in tablet dosage forms was simple, rapid, accurate, precise, specific, robust and economical. The mobile phase is simple to prepare and economical reliable, sensitive and less time consuming.

Since the system suitability studies as well as its validation studies have shown satisfactory as well, it is deduced that the simple and short proposed method be most useful for analysis purpose.

CONCLUSION:

The present study was validated as per the ICH guidelines.

From the comparative study, it was inferred that the method is simple, specific, precise, linear, sensitive, and also system suitability. The results obtained on the validation parameter met the respective acceptance criteria.

The method was found to have suitable application in routine laboratory analysis and with high degree of accuracy and precision.

From the comprehensive validation conducted, it was concluded that the method is stable and could be used throughout shelf life of the drug.

VI. CHROMATOGRAM

SPECIFICITY :

Fig : 6 Olanzapine and Fluoxetine HCl

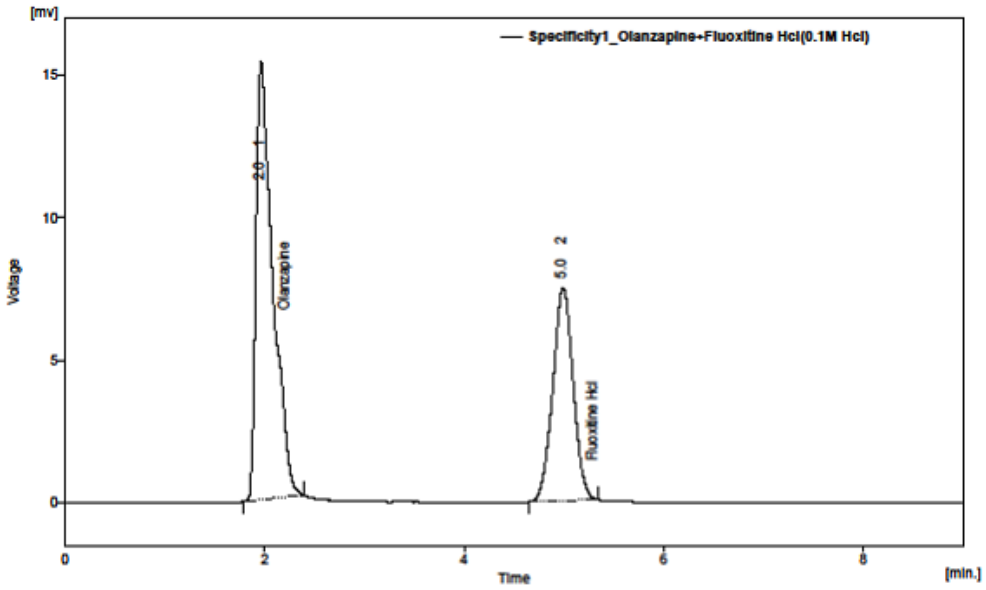
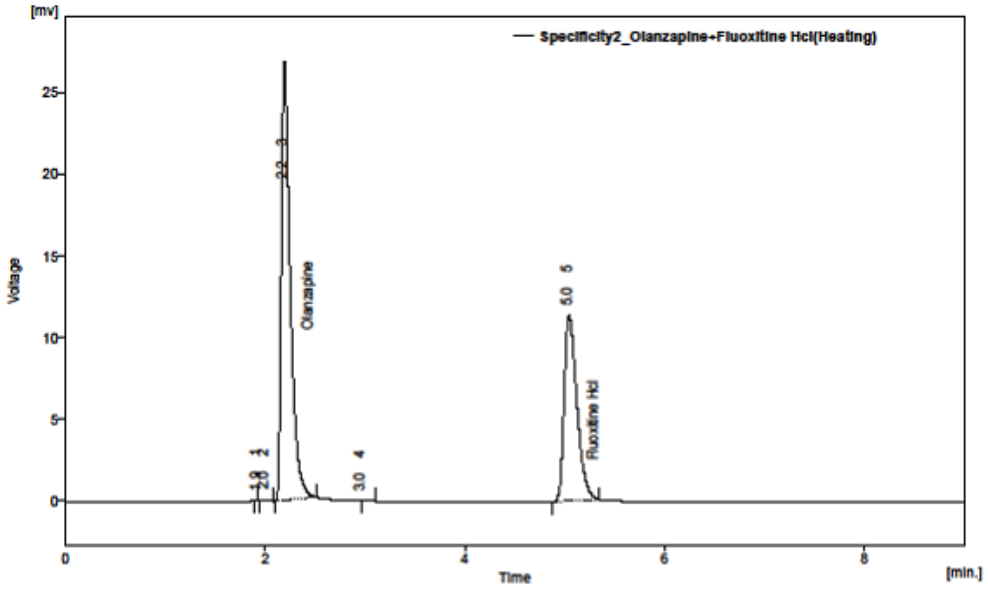


Fig : 7 Olanzapine and Fluoxetine HCl



LINEARITY

Fig : 8 Olanzapine(2 ppm) and Fluoxetine HCl(4 ppm)

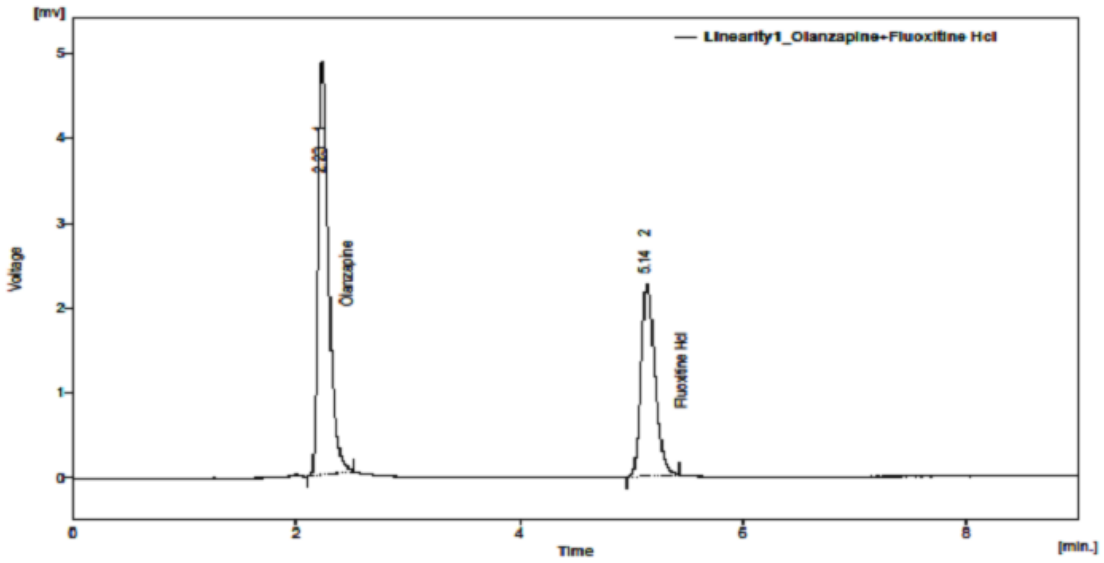


Fig : 9 Olanzapine (4 ppm) and Fluoxetine HCl(8 ppm)

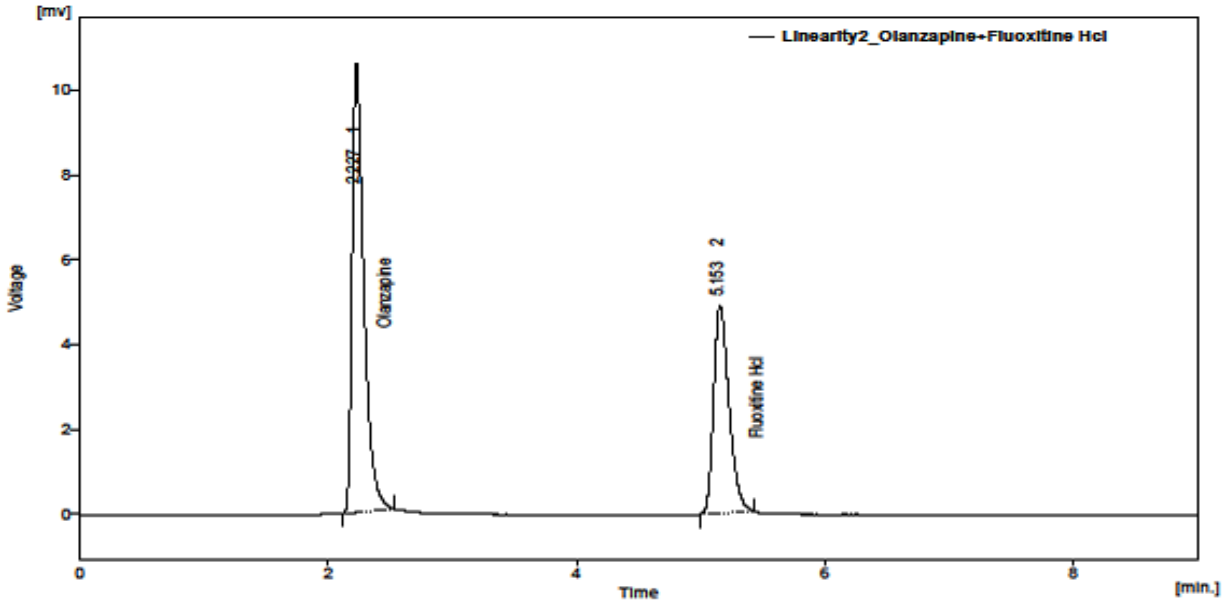


Fig : 10 Olanzapine(6 ppm) and Fluoxetine HCl(12 ppm)

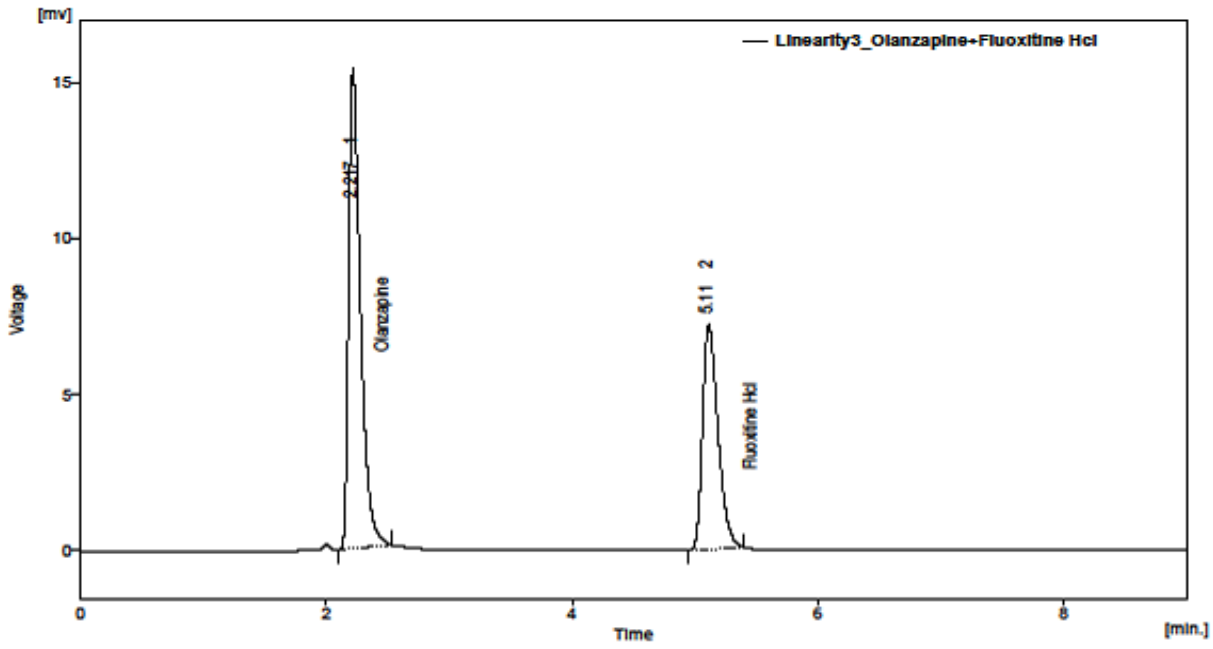


Fig : 11 Olanzapine(8 ppm) and Fluoxetine HCl(16 ppm)

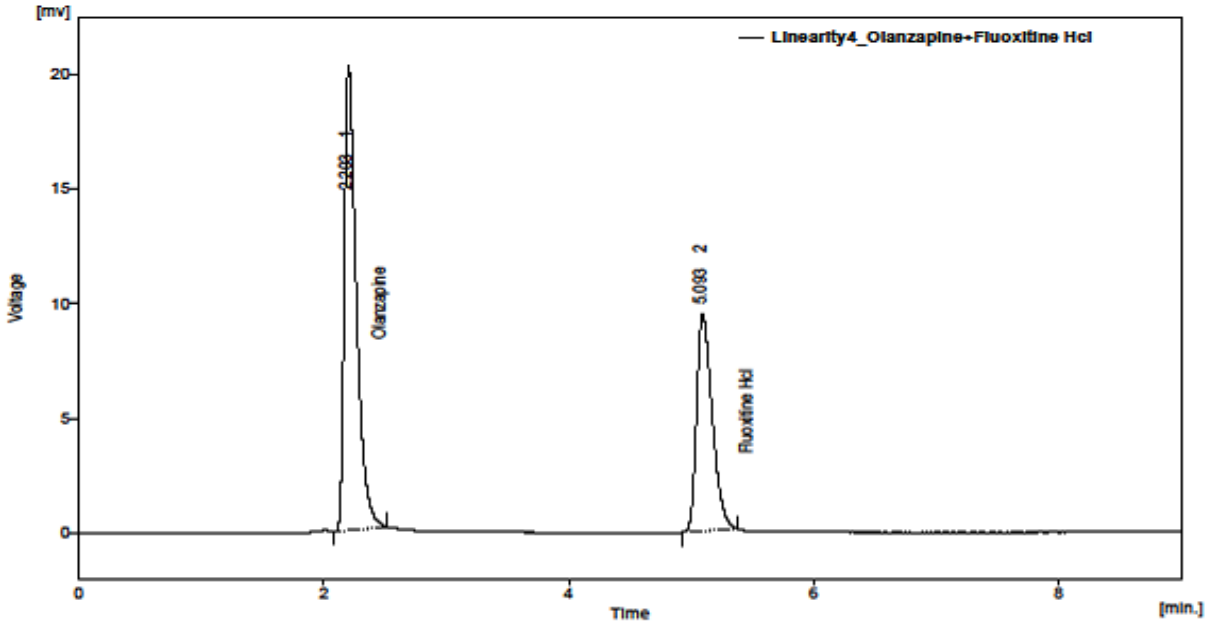


Fig : 12 Olanzapine (10 ppm) and Fluoxetine HCl (20 ppm)

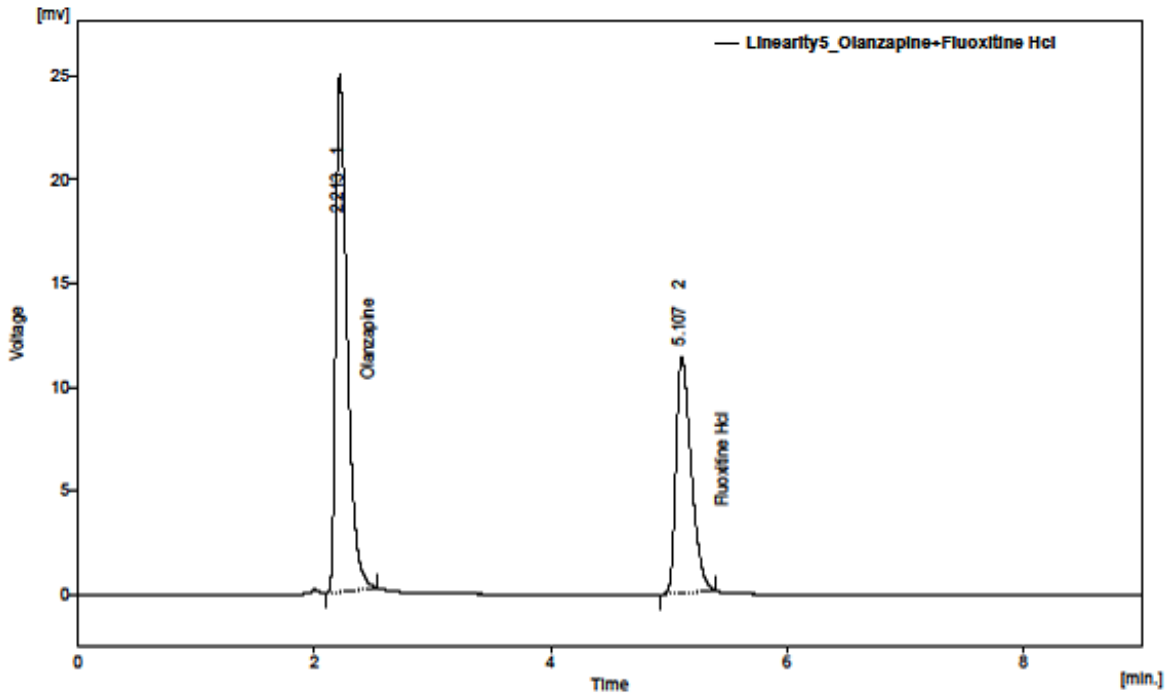
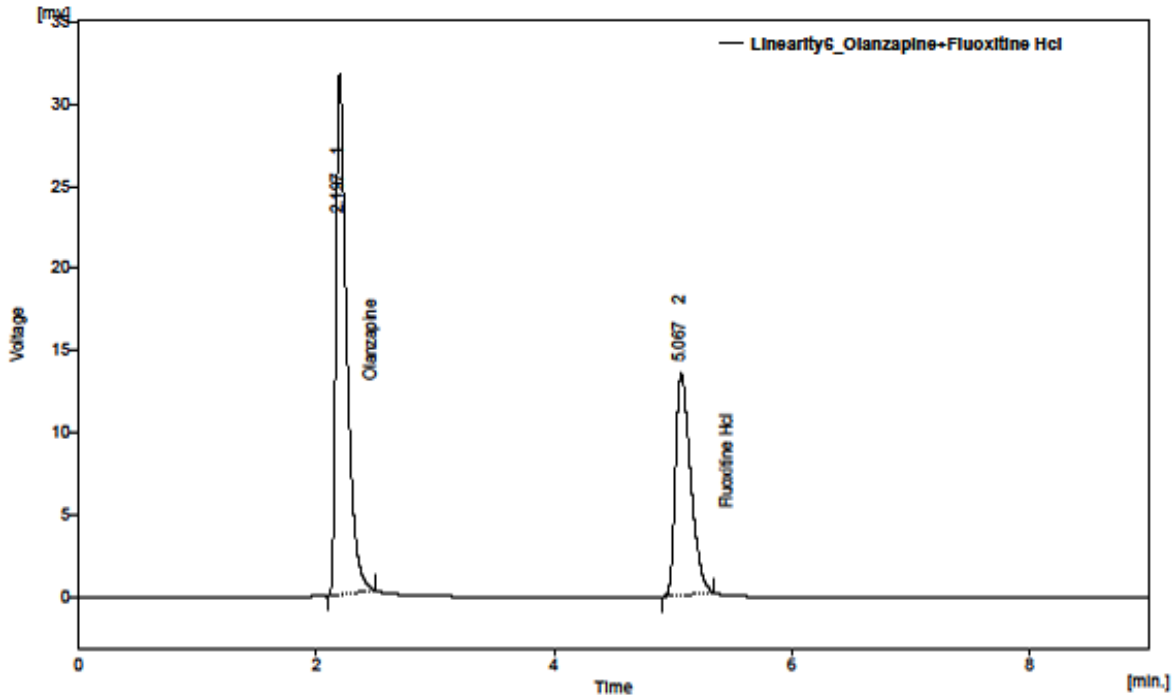


Fig : 13 Olanzapine (12 ppm) and Fluoxetine HCl (24 ppm)



PRECISION

Fig : 14 Olanzapine (10 mcg) and Fluoxetine HCl (20 mcg)

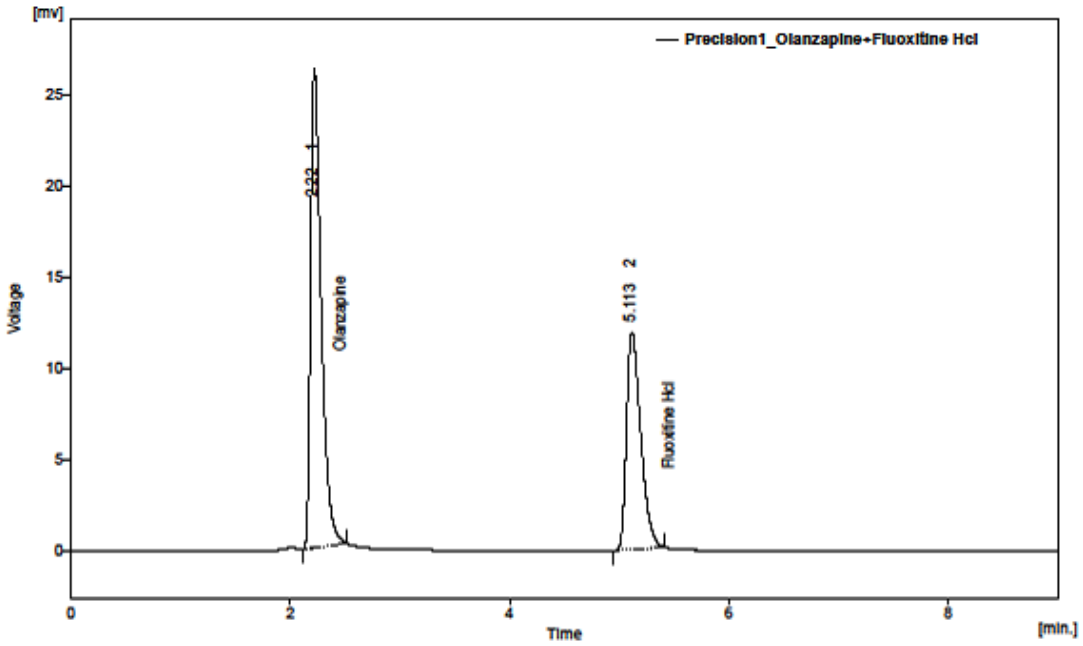


Fig : 15 Olanzapine (10 mcg) and Fluoxetine HCl (20 mcg)

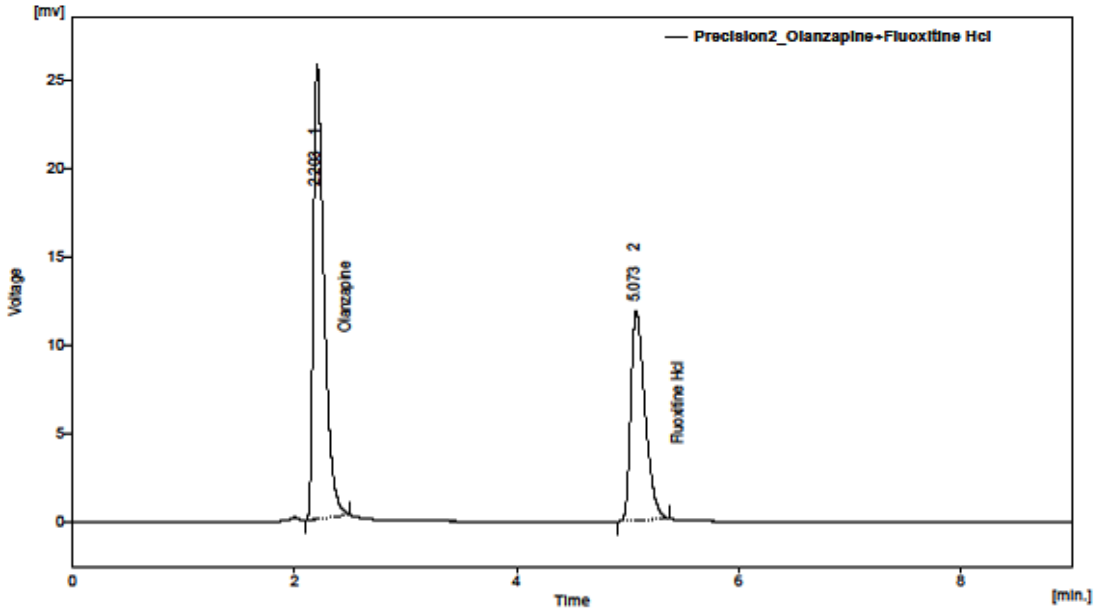


Fig : 16 Olanzapine (10 mcg) and Fluoxetine HCl (20 mcg)

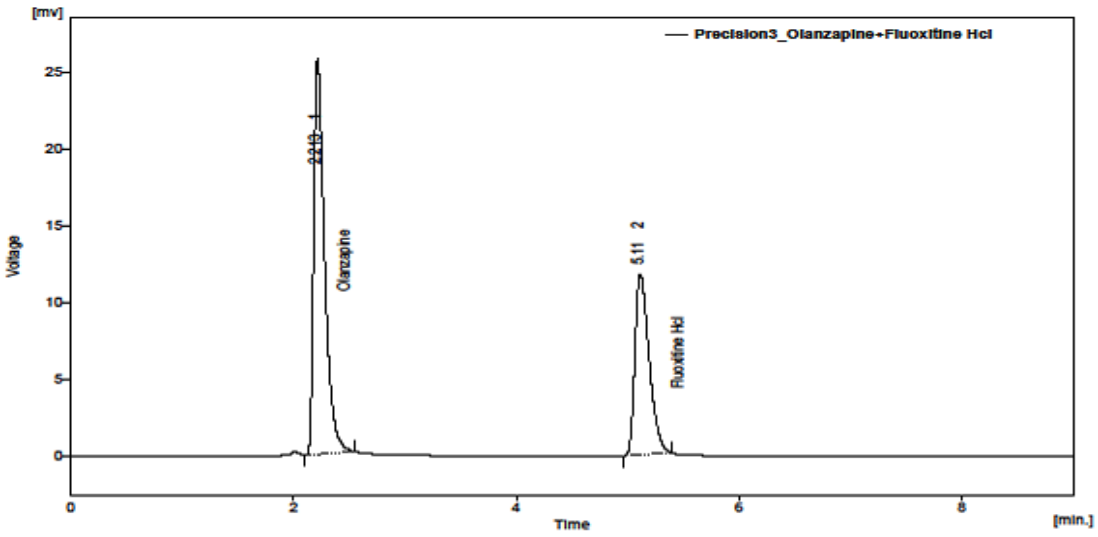


Fig : 17 Olanzapine (10 mcg) and Fluoxetine HCl (20 mcg)

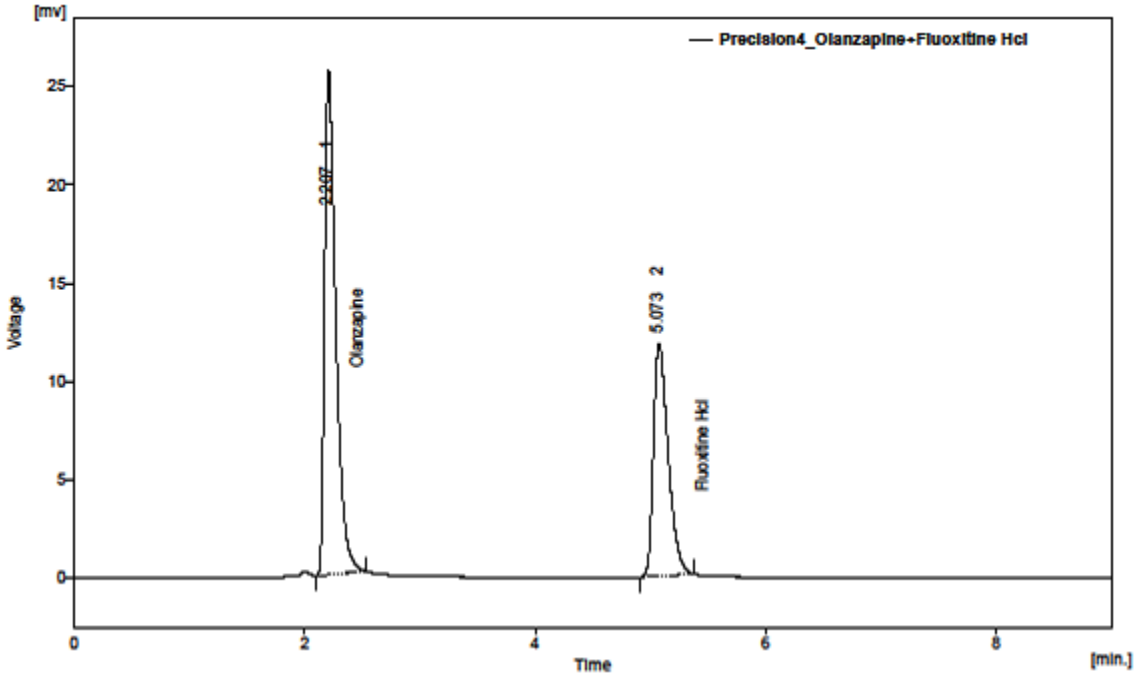
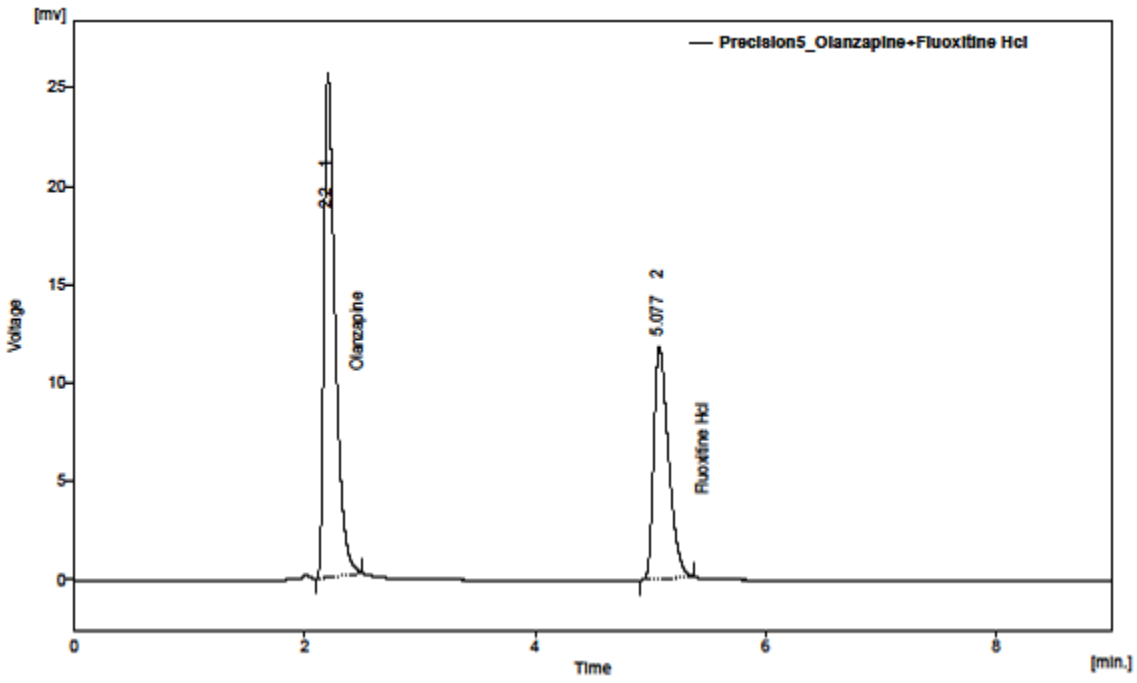


Fig : 18 Olanzapine (10 mcg) and Fluoxetine HCl (20 mcg)



ASSAY

Fig : 19 Olanzapine (10 mcg) and Fluoxetine HCl (20 mcg) (Sample)

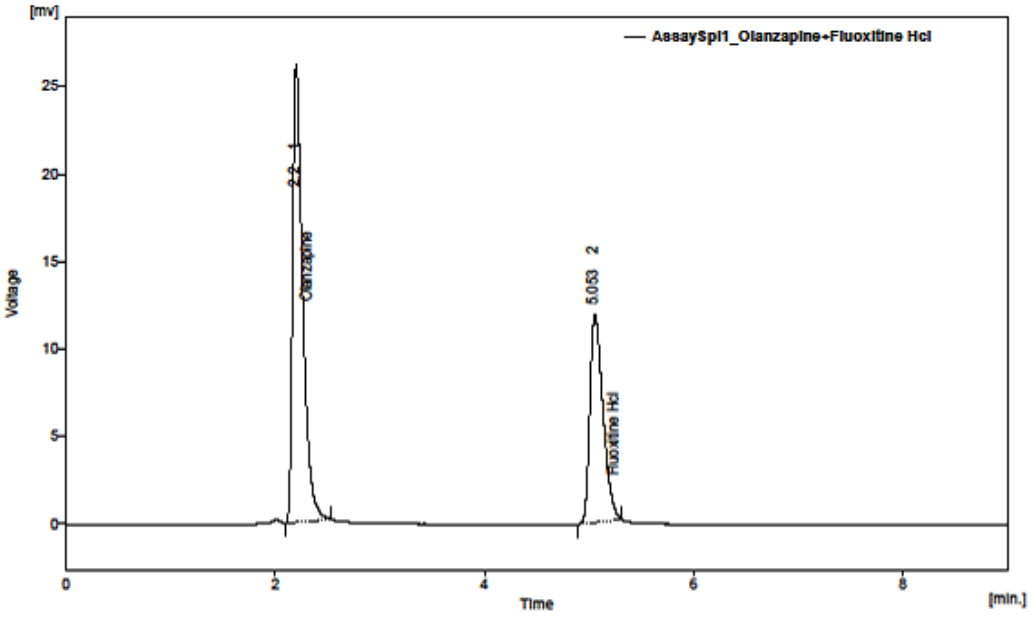


Fig : 20 Olanzapine (10 mcg) and Fluoxetine HCl(20 mcg) (Std)

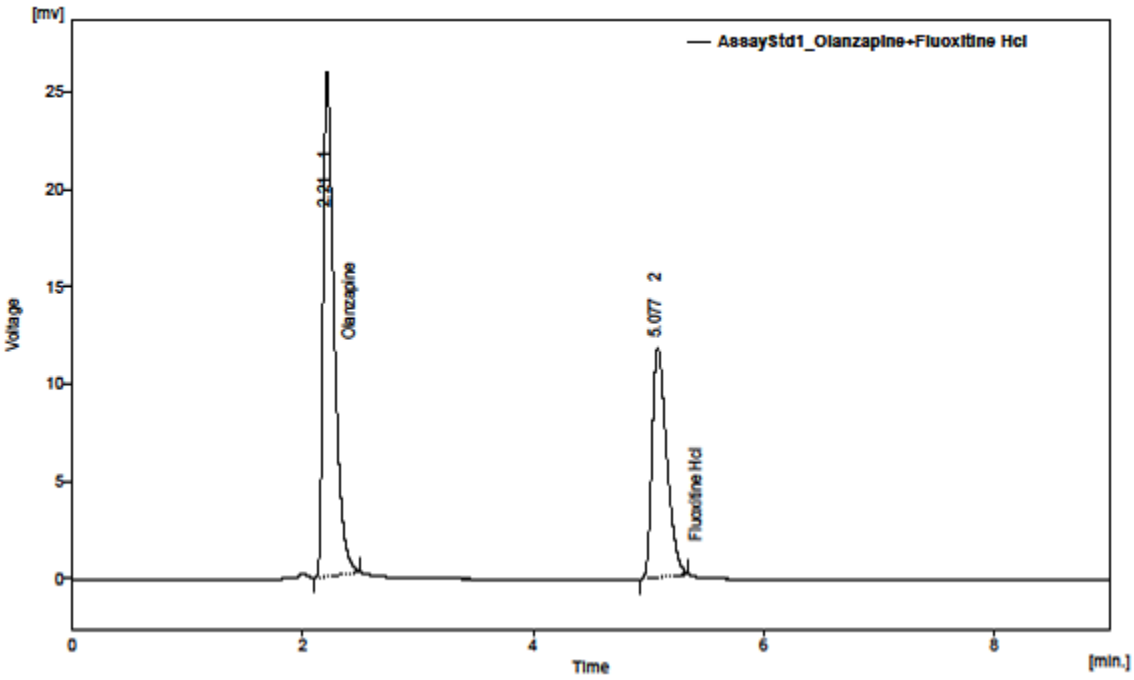


Fig : 21 Olanzapine (10 mcg) and Fluoxetine HCl (20 mcg) (Sample)

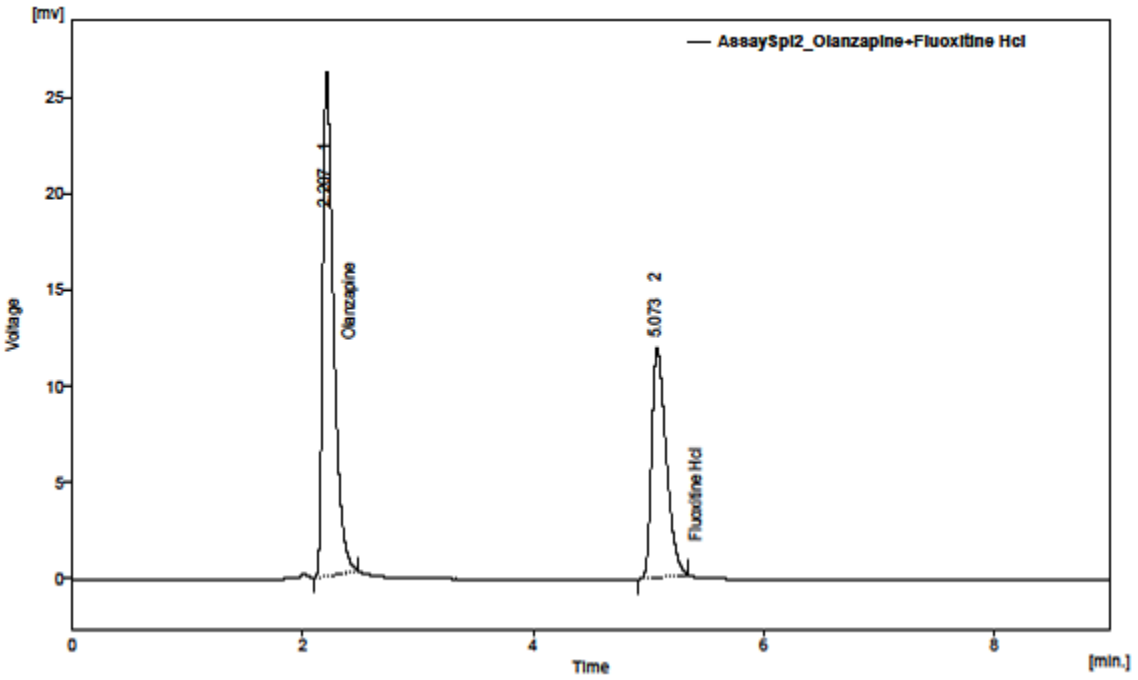


Fig : 22 Olanzapine (10 mcg) and Fluoxetine HCl (20 mcg) (Std)

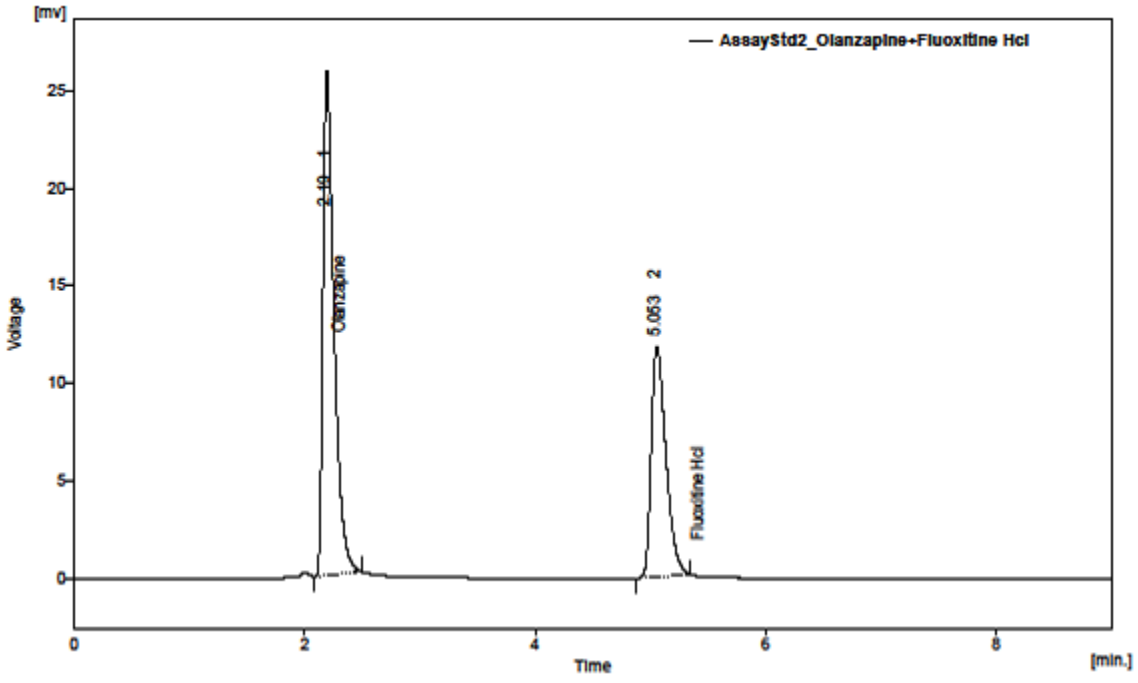
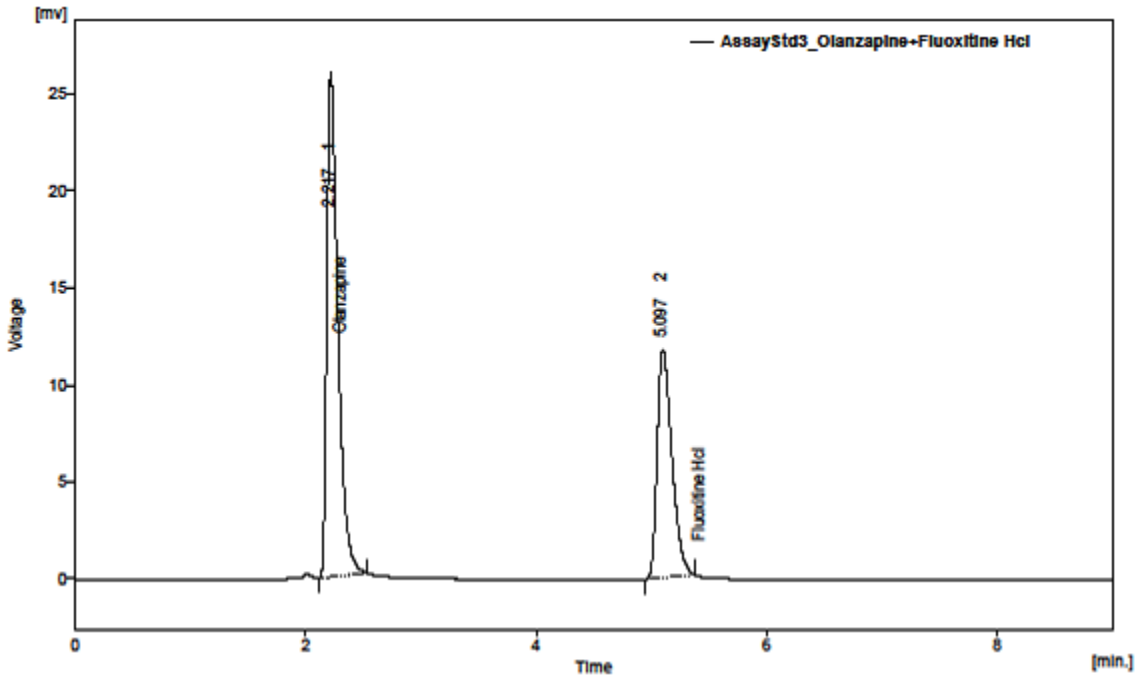


Fig : 23 Olanzapine (10 mcg) and Fluoxetine HCl (20 mcg) (Std)



ACCURACY

Fig : 24 Olanzapine (9 mcg) and Fluoxetine HCl (18 mcg)

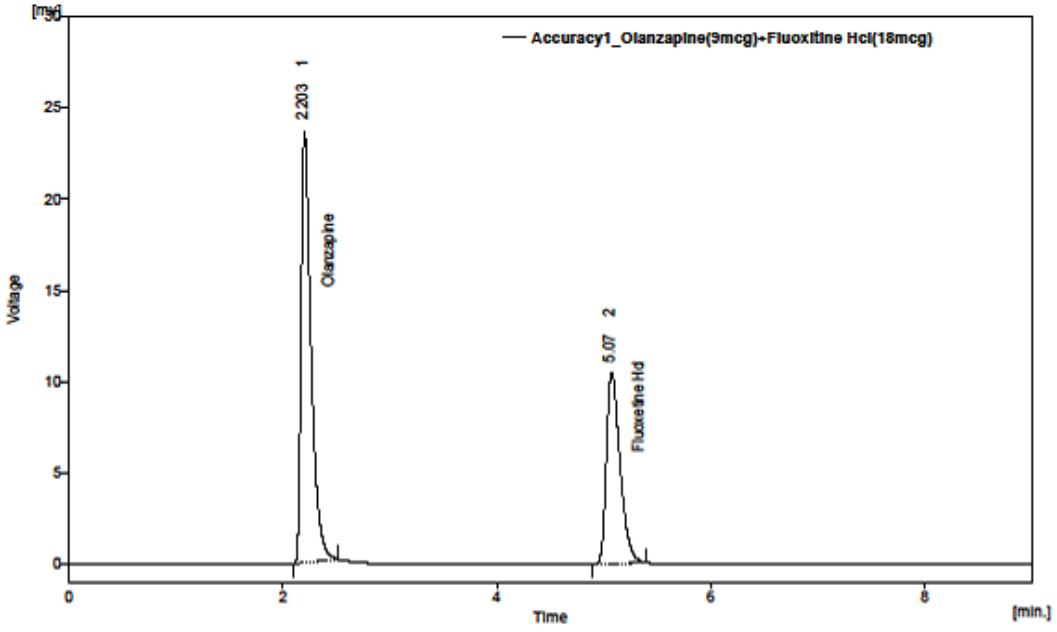


Fig : 25 Olanzapine (11 mcg) and Fluoxetine HCl (22 mcg)

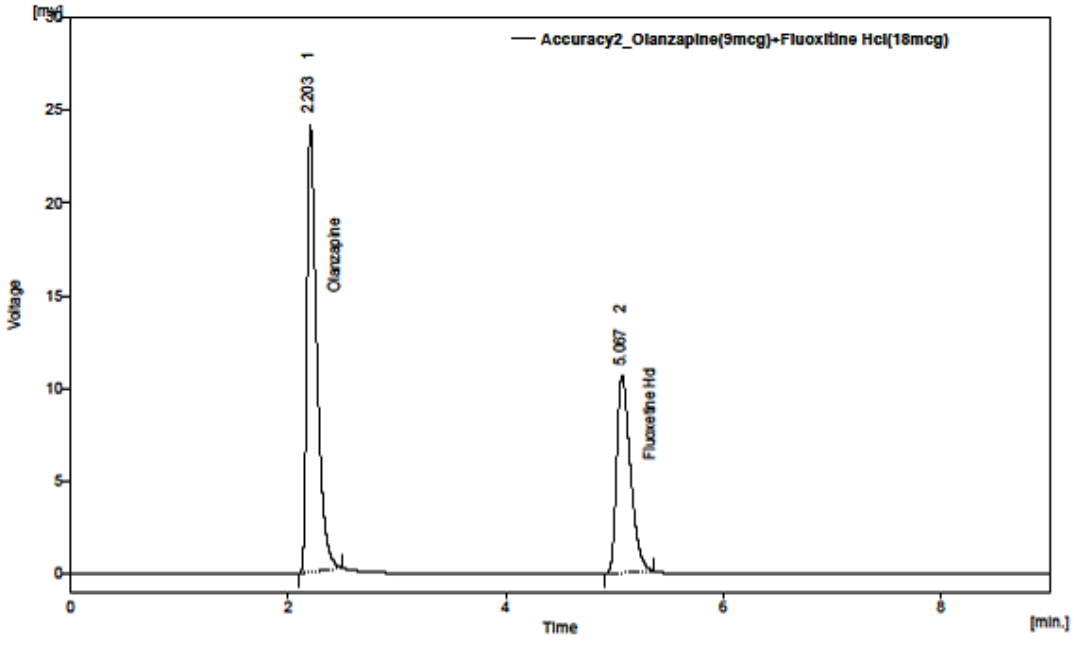


Fig : 26 Olanzapine (13 mcg) and Fluoxetine HCl (26 mcg)

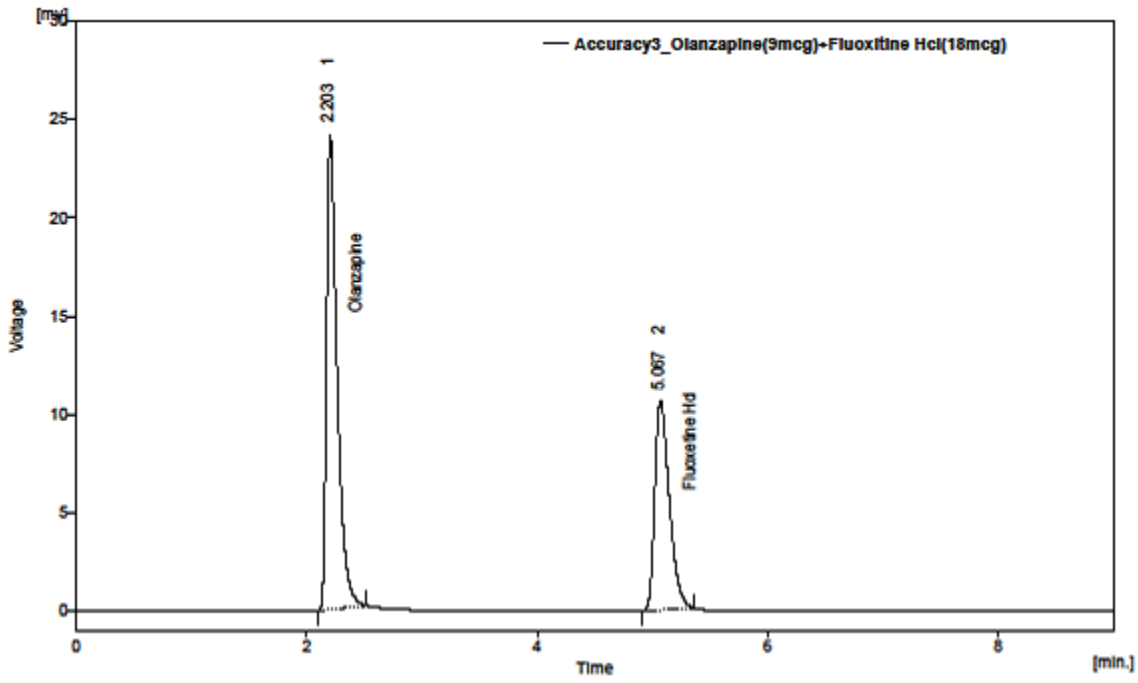


Fig : 27 Olanzapine (10 mcg) and Fluoxetine HCl (20 mcg)(Spiking standard)

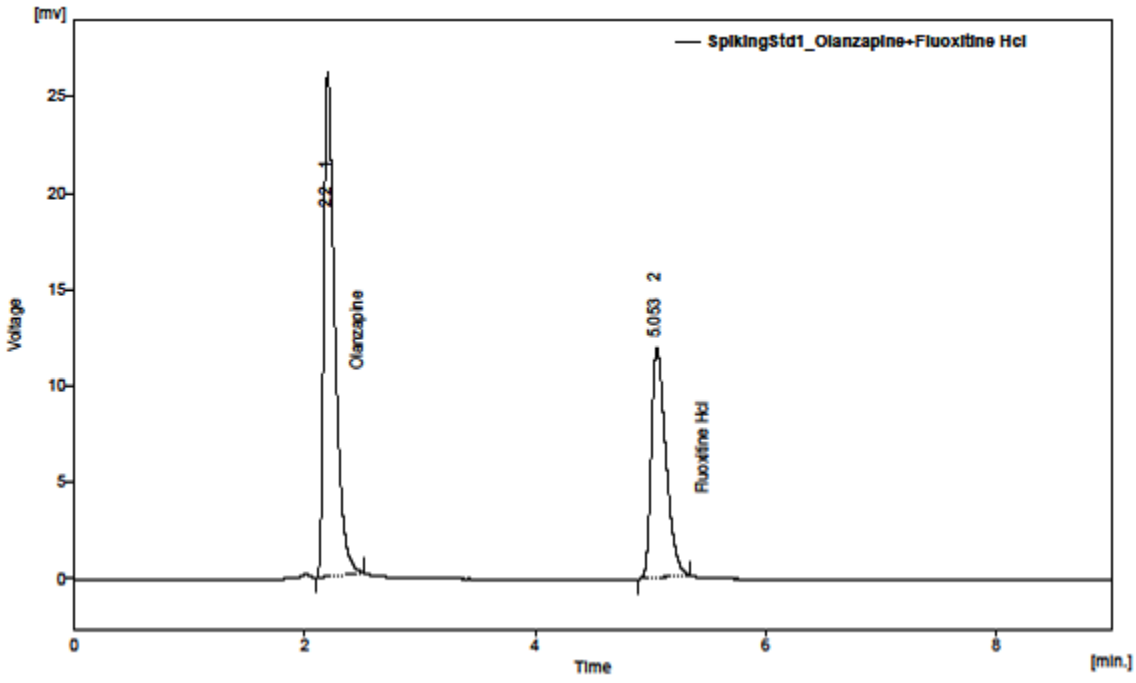


Fig : 28 Olanzapine (10 mcg) and Fluoxetine HCl (20 mcg)(Spiking standard)

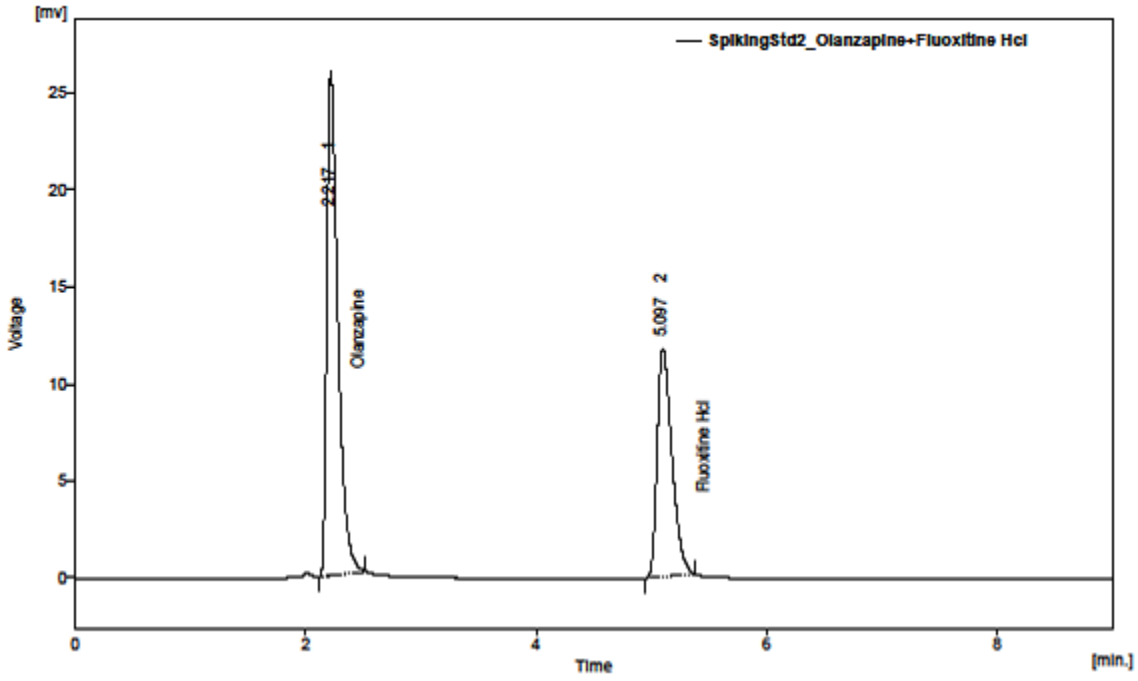
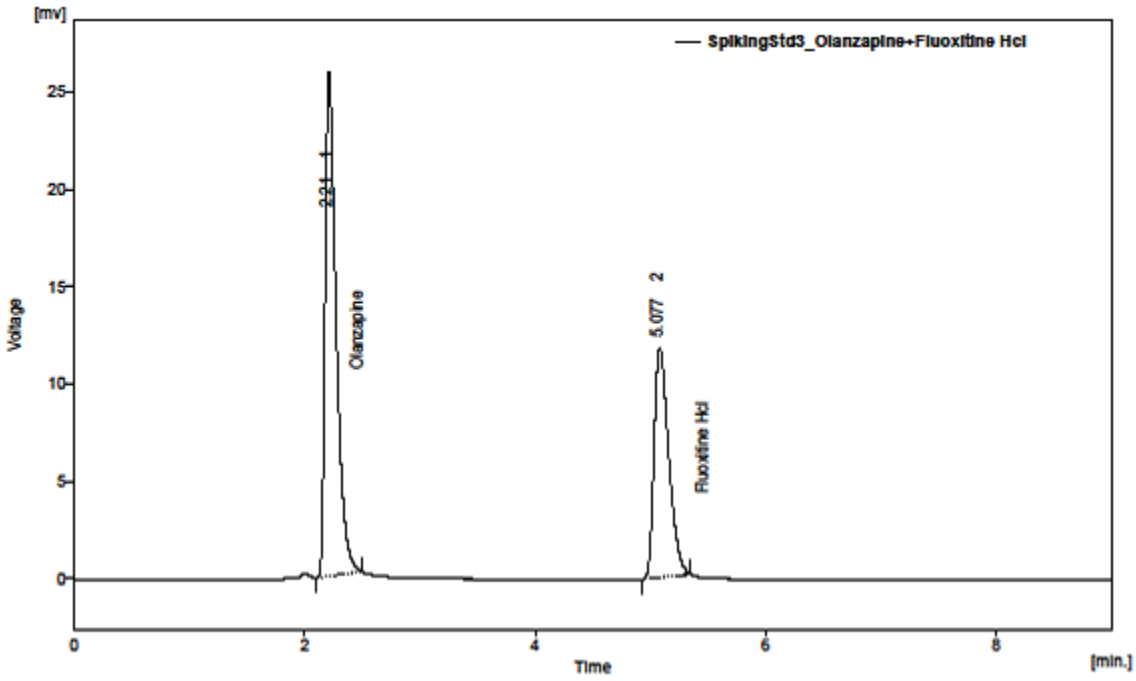


Fig : 29 Olanzapine (10 mcg) and Fluoxetine HCl (20 mcg)(Spiking standard)



METHOD PRECISION

Fig : 30 Olanzapine (10 mcg) and Fluoxetine HCl (20 mcg)

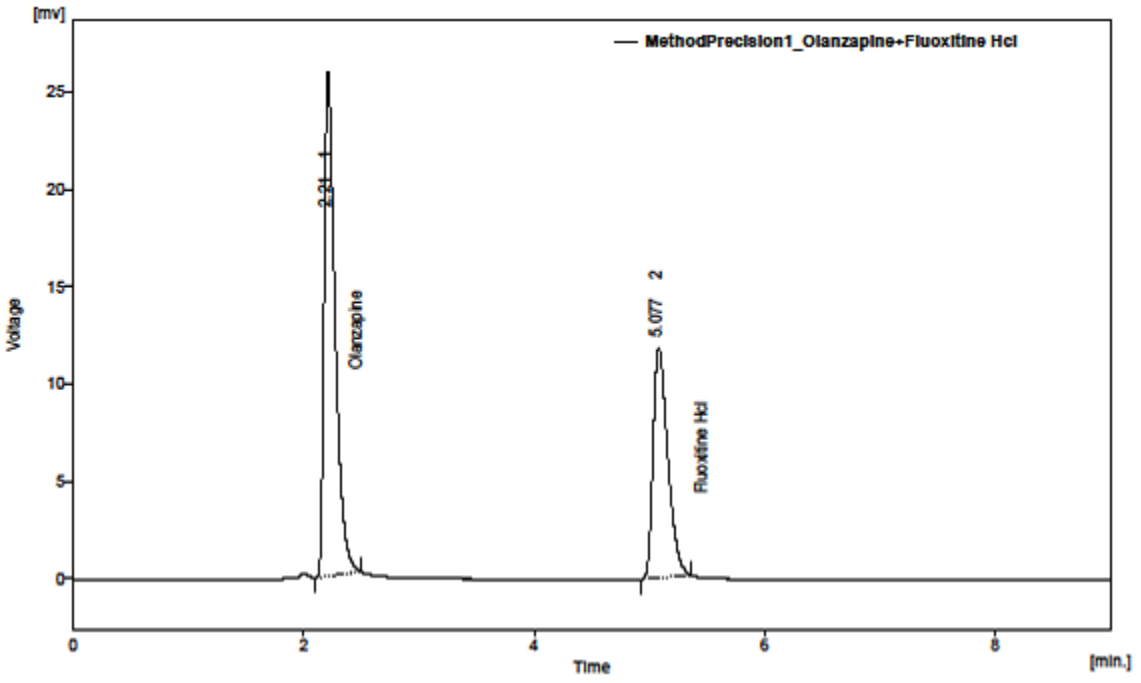


Fig : 31 Olanzapine (10 mcg) and Fluoxetine HCl (20 mcg)

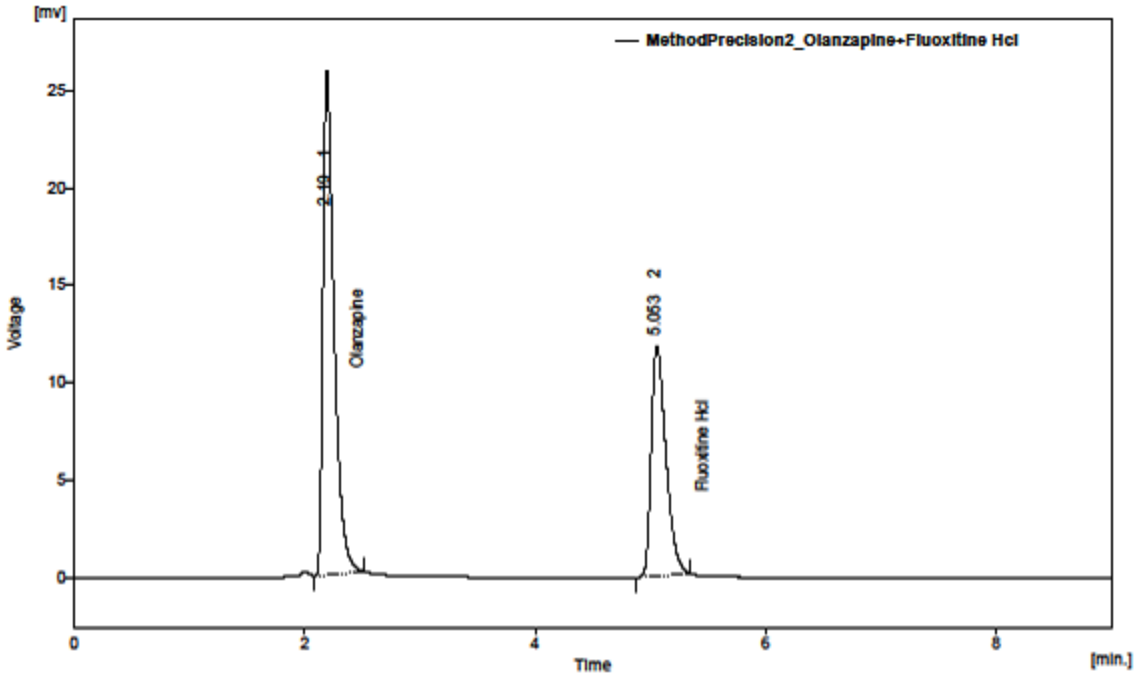


Fig : 32 Olanzapine (10 mcg) and Fluoxetine HCl (20 mcg)

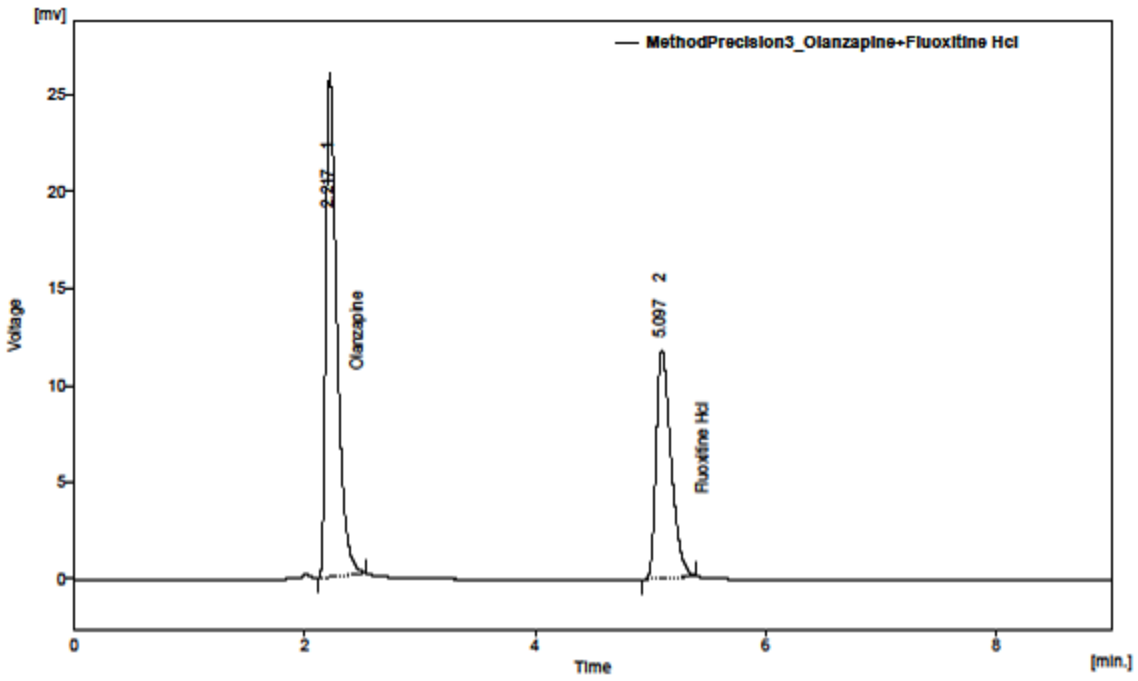


Fig : 33 Olanzapine (10 mcg) and Fluoxetine HCl (20 mcg)

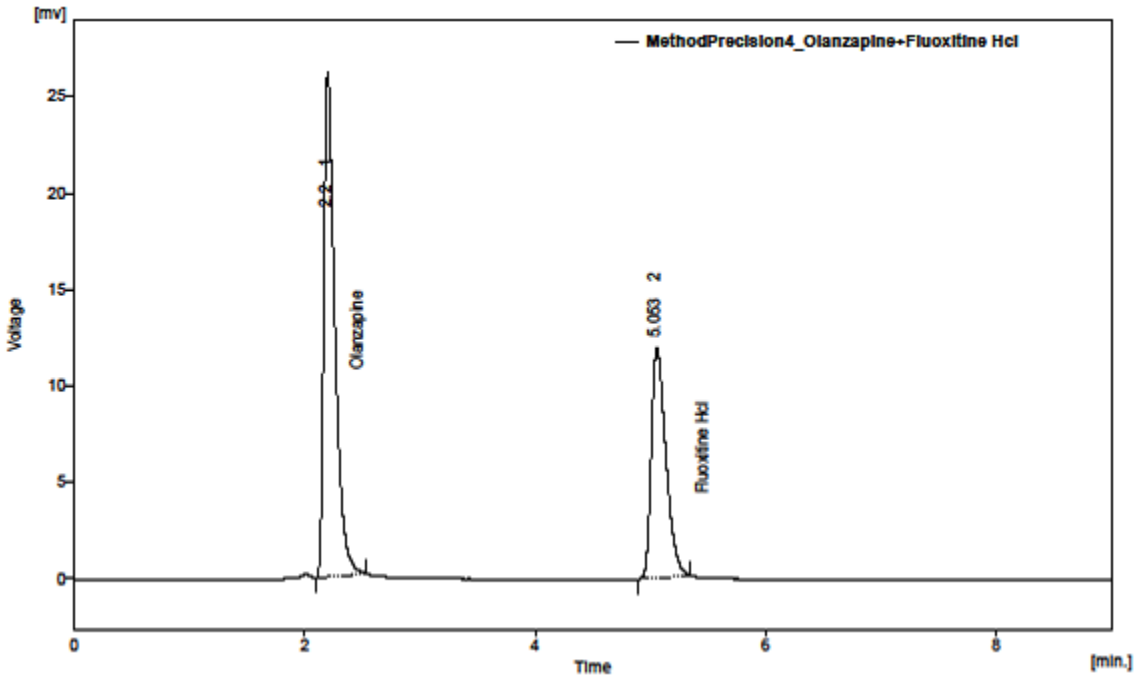
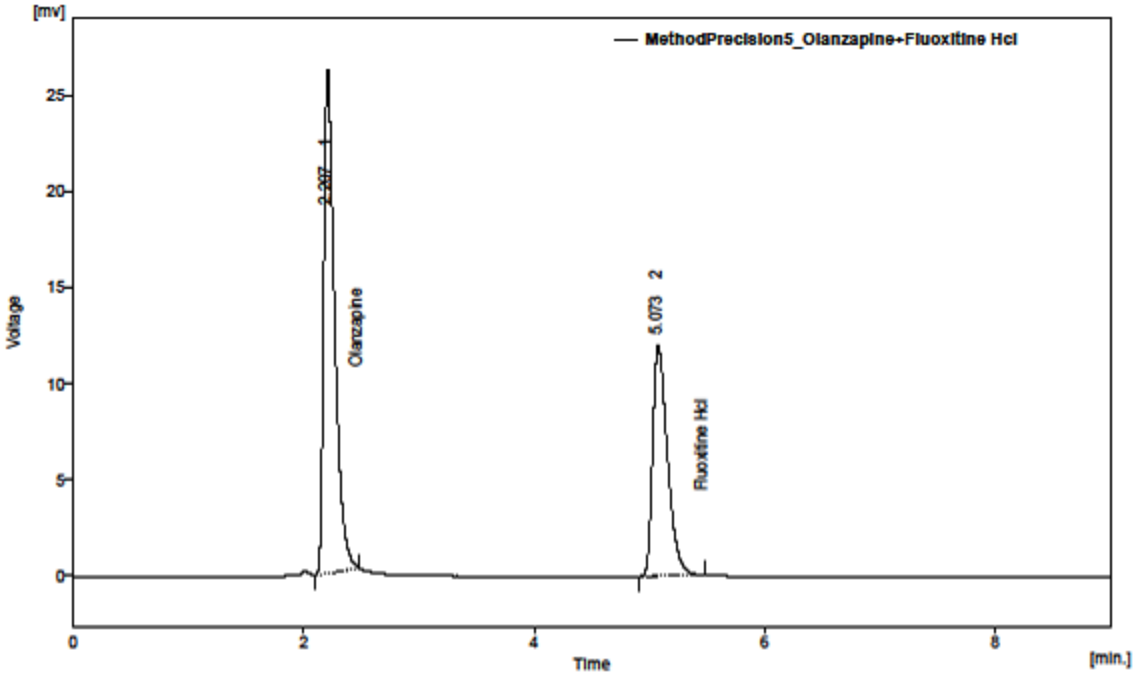


Fig : 34 Olanzapine (10 mcg) and Fluoxetine HCl (20 mcg)


[mv]
MethodPrecision5_Olanzapine+Fluoxitine Hcl
25
20
15
10
5
0
Voltage
2.307 1
Olanzapine
5.073 2
Fluoxitine Hcl
0
2
4
6
8
Time
[min.]

RUGGEDNESS

Fig : 35 Olanzapine (10 mcg) and Fluoxetine HCl (20 mcg)

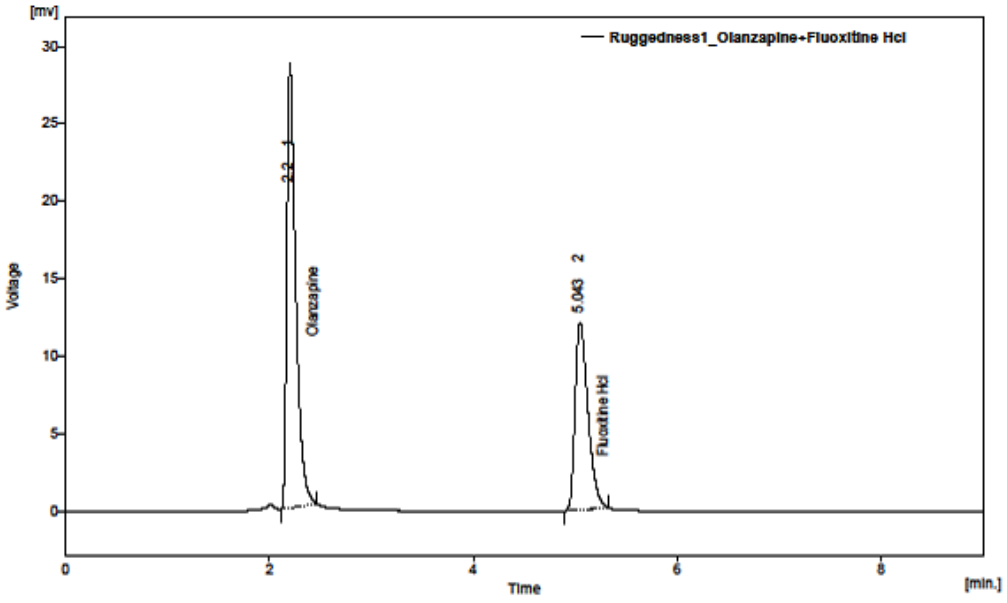
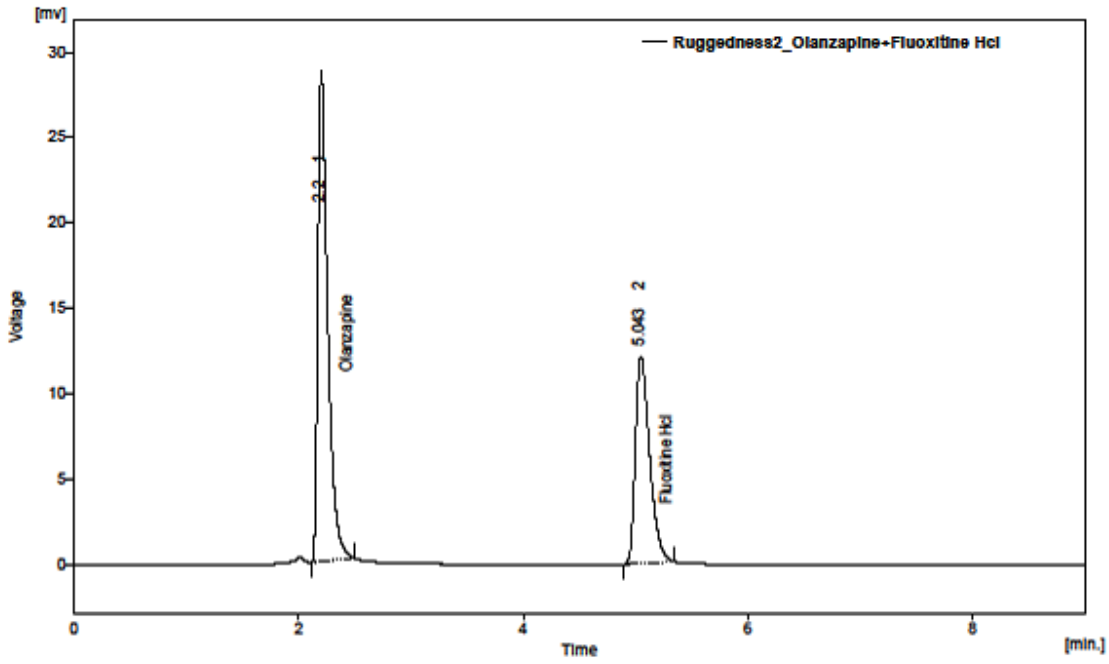


Fig : 36 Olanzapine (10 mcg) and Fluoxetine HCl (20 mcg)



ROBUSTNESS

Fig : 37 Olanzapine (10 mcg) and Fluoxetine HCl (20 mcg)

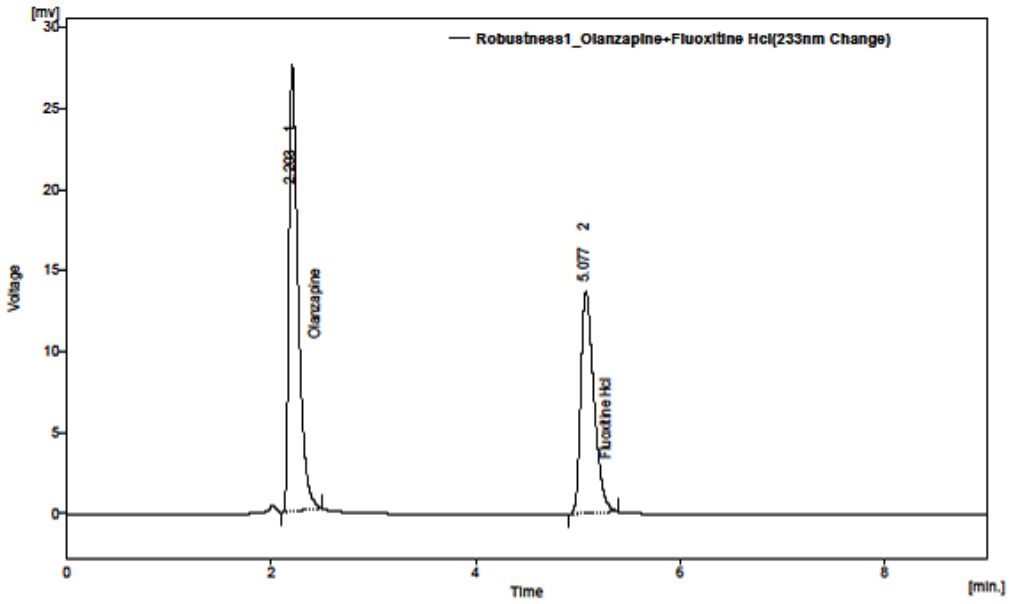


Fig :38 Olanzapine (10 mcg) and Fluoxetine HCl (20 mcg)

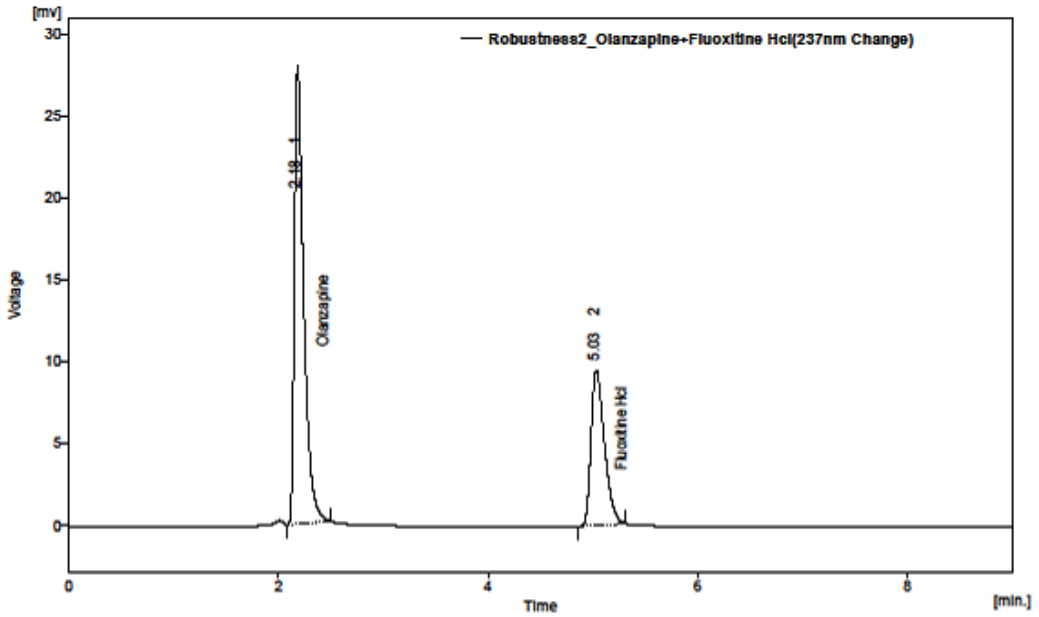


Fig : 39 Olanzapine (10 mcg) and Fluoxetine HCl (20 mcg)

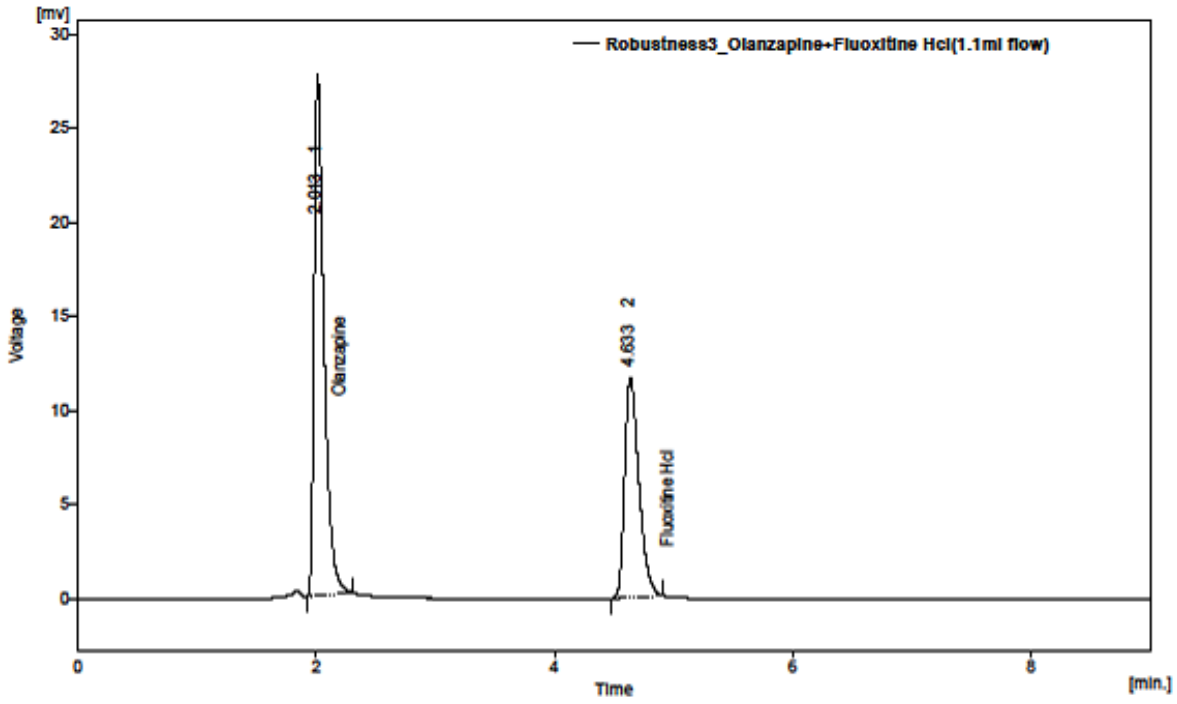
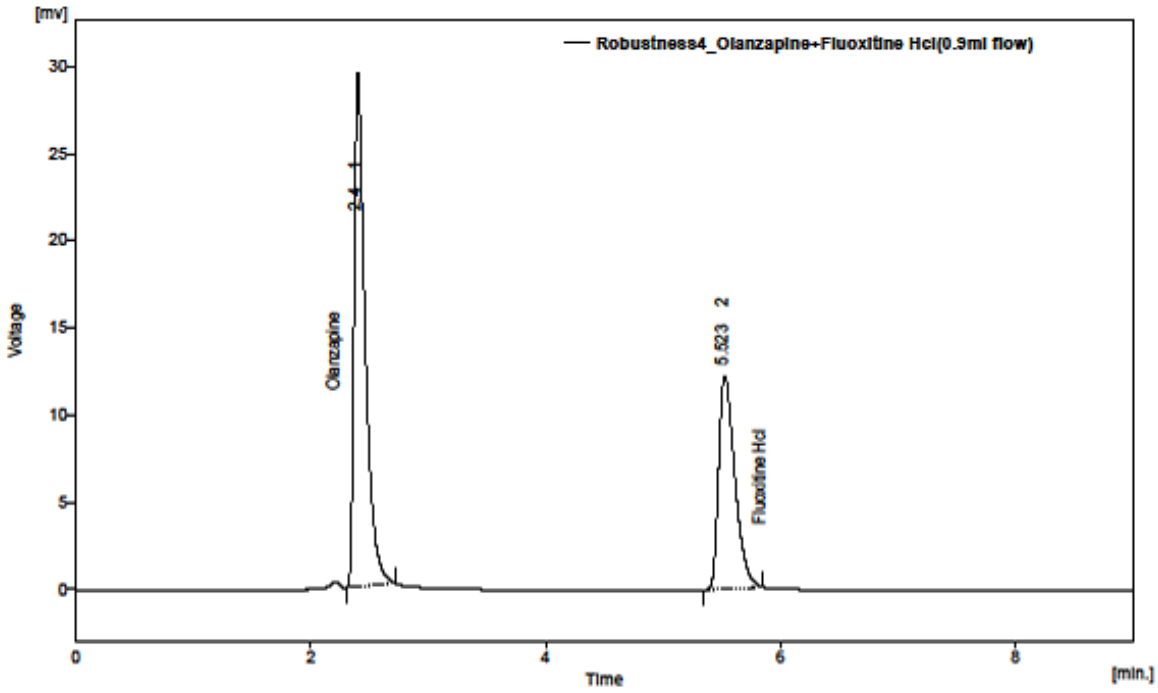


Fig : 40 Olanzapine (10 mcg) and Fluoxetine HCl (20 mcg)



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